OLEARIA PHLOGOPAPPA: ASPECTS OF CLONAL CULTIVATION AND ESSENTIAL OIL CHARACTERISATION

by

Annette

VALERIE DRAGAR (B.Agr.Sc., M.Agr.Sc.) Tas.

Submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

UNIVERSITY OF TASMANIA

HOBART

APRIL, 1990

This thesis contains no material which has been accepted for the award of any other degree or diploma in any other university, and, to the best of my knowledge, contains no copy or paraphrase of material previously published or written by any other person, except where due reference is made in the text of the thesis.

V.A. DRAGAR

UNIVERSITY OF TASMANIA

HOBART

APRIL, 1990

CONTENTS

ACKNOWLEDGEMENTS

I would like to express my gratitude to Dr. R.C. Menary for his supervision, guidance and enthusiasm throughout the course of this project.

My thanks are also extended to Mr. N.W. Davies, Dr. M. Ridd, and Mr. W. Jablonski of the Central Science Laboratory, University of Tasmania, for assistance with the determination of mass spectra, the routine gas-chromatographic work and the use of the scanning electron microscope, respectively.

Dr. B.M. Potts assisted with the statistical analysis of agronomic data for which I owe him many thanks.

I wish to thank Mr. W. Peterson, Mr. G. Richardson, Mr. C.D. Read, Mr. M.G. Mason and Mr. M. Dragar for their willingness to help with various technical and maintenance problems that occurred all too often.

The award of a Commonwealth Government Postgraduate Award is gratefully acknowledged.

A special thanks is due to Dr. C. Dragar for his assistance with the chemistry of Olearia essential oils.

Thank you Roman.

Finally, sincere thanks are due to my family and friends. Their moral and practical support, their patience and their understanding was invaluable throughout this long endeavour.

OLEARIA PHLOGOPAPPA: ASPECTS OF CLONAL CULTIVATION AND ESSENTIAL OIL CHARACTERISATION

SUMMARY

The species *Olearia phlogopappa* was found to exhibit variation in both form and essential oil properties in populations around Tasmania. Six locations, Great Lake, Paradise Plains, Mount Wellington, Eaglehawk Neck, Elephant Pass and Buckland, were surveyed for a plant which yielded maximal quantities of organoleptically suitable essential oil from each site.

Some preliminary morphological, dry matter and infra-red photographic studies were carried out which revealed a positive relationship between the rate of dry matter accumulation and height measurements. This, together with a small scale seasonal variation trial led to the development of multi-location trials.

A rank order of clones was established on the basis of growth rate and oil yield. The major source of oil appeared to be the juvenile leaves in all cases. The effect of harvest was studied at two trial sites. A combination of a pruning trial and seasonal variation trials led to the recommendation that *Olearia phlogopappa* clones should be grown in a hedgerow and harvested in late summer. The plants can be cut to a height of approximately 30 cm, and the regenerative growth may be harvested a second time in August. In this way, the maximum oil yield may be obtained.

The characterisation of the essential oil of each of six clones was carried out using a combination of gas chromatography/mass spectrometry, nuclear magnetic resonance spectroscopy, high performance liquid chromatography and Fourier transform infra-red techniques.

The essential oil was pre-fractionated by means of a simple silica gel column. Through the use of this technique, fractions were obtained for further characterisation work. The separation occurred on the basis of polarity, so that hydrocarbons, oxygenated compounds and alcohols were obtained in separate fractions. This simplified the hplc purification and subsequent identification by nmr of several important components. Some 2D nmr experiments, in

addition to standard ^{1}H and ^{13}C nmr techniques were used. The major components identified were as follows:
α-pinene, β-pinene, cineole, linalool, α-terpineol, caryophyllene, germacrene-D, bicyclogermacrene, spathulenol, α-eudesmol, β-eudesmol, γ-eudesmol, liguloxide, caryophyllene oxide and kessane.

In addition to the above components, there are some 50 minor components present in the oils, some of which contribute to the characteristic tomato, exotic fruity odour of these products.

Changes in oil composition were suspected to be occurring during the steam distillation process, and these were investigated. The essential oil released from the plant material was compared to the release of volatiles from a solvent extract.

The diversity of the clonal material was investigated concentrating on differences in several morphological features and growth rates. The seasonal variation of essential oil in the leaves was monitored and the greatest quantities of oil were found to be present in late summer, early autumn. The bulk of the oil is located in the current seasons growth, rather than in matured leaves. The quality of the oil was also observed to change through the monitoring of chemical composition.

Organoleptically, the oils from the six clones under consideration were markedly different from one another. For instance, the desirable spicey, tomato-like properties were predominantly found only in one clone. This odour characteristic was also present in the other oils but was masked or over-ridden by other exotic fruity notes. In other cases, native bush, floral, or citrus notes were predominant in the oil.

The major components of the oil were identified as germacrene-D, bicyclogermacrene and spathulenol. In some instances, β-eudesmol or caryophyllene comprised the greatest part of the oil. However, the greatest contribution to the overall characteristic spicey odour was made by kessane and liguloxide. Minor components were not identified, but are also likely to be responsible for the complex aroma of the oil.

This work makes a contribution to the body of knowledge relating to essential oils in endemic species. The uniqueness of Olearia oil is illustrated by its odour, and by its adaptability to a cropping situation. The commercial production of a natural

essence such as this one heralds the inception of a potentially viable new industry.

I. INTRODUCTION

"...In form, bearing, scent and taste, plants are so varied that with a little practice and some knowledge of botany, one cannot confuse even the most closely related species...."

M. Bucquet 1773.

"...Before all else it is proper to use care both in the storing up and in the gathering of herbs each at its due season, for it is according to this that medicines either do their work, or become quite ineffectual..."

Dioscorides ca. A.D. 50.

"...The active substances contained in plants vary, in at times considerable proportions, according to the age of the plant, the time of picking, the nature of the soil and the climate..."

J. Leclerc 1657-1736.

The knowledge that certain members of the plant kingdom possess healing, restorative, stimulative, sedative, haemostatic, anti-spasmodic and other beneficial properties has been with mankind for centuries. The desire to determine the mechanism of action of these 'herbs' has led to the isolation of the essences of these plants - the essential oils.

An essential oil has been defined as a volatile material derived by physical processes from odorous plants of a single botanical form and species, with which it agrees in name and odour (Arctander S., 1960). In general, an essential oil is derived by steam distillation or expression. Expression uses mechanical force, while distillation may be by water, steam or a combination of the two.

In comparison, a solvent extract also contains volatile substances, but in addition, carries with it materials such as solids and waxes, which are not readily removed from the plant material by steam distillation.

Extracts derived from plants have a multitude of established and potential uses. Oils are used for medicinal purposes; for instance, eucalyptus oil finds applications ranging from cough

formulations to liniments, and tea-tree oil has bactericidal properties.

Another field of application that is as vast as the imagination is in the enhancement of food flavours. Citrus oils are the most frequently used for confectionery, along with peppermint, which is also found in products such as toothpaste. Spice oils are added to many pre-cooked or frozen foods to bring out or replace flavours that have dissipated or were perhaps lost during processing.

The other major area where oils are found is in the fragrancing of countless products from air fresheners to disinfectants. The range of applications in everyday household items seems vast; every product that is used for cleaning, scouring, disinfecting or deodorising relies on an attractive odour for acceptance. The limits of the fragrance application are reached when essential oils are taken by expert hands and transformed into delicate, exotic, sophisticated or otherwise distinguished perfumes. When individual fragrances (or flavours) are blended into a complex mixture that evokes precisely the desired sentiment or reaction, it is indeed 'art'.

Essential oils may be obtained from leaves and stems (for example, peppermint oil), while lovage bears its essential oil in the roots. Alternatively, extracts may be derived from the flowers as in the case of rose and jasmin. There are also instances when other plant organs contain oil. Among these are caraway, cardamom, dill, fennel, parsely etc. which have oil-laden seeds whereas clove oil and cassis extracts are derived from buds.

New sources of essential oils are continually being sought, especially complex ones with properties which are difficult to duplicate synthetically. Certain woody type oils like vetiver or sandalwood are valued because attempts to synthesize their odours have failed (Heath H.B., 1978).

Areas remain around the world, where the native vegetation is incompletely researched, and the essential oils of odoriferous plants remain uncatalogued; their potential remains hidden. According to many researchers, the endemic flora of Australia contains many plant species that may be of use to society, either medicinally or otherwise.

Similarily in Tasmania, the potential to discover a useful essential oil plant is great, since the total amount of research in

this area has been minimal to date.

The selection and cultivation of an indigenous plant variety for essential oil production is rare in Australia. One exception is Boronia, whose flowers are used to produce a very delicate, highly prized extract. However, in terms of a herb or shrub which has oil bearing leaves and is processed by steam distillation, there is not a single example. Eucalyptus species are grown commercially to some extent, but they, like tea-tree are not often cultivated. Rather, the plant material is collected from the bush and natural regeneration processes are relied upon.

Despite the lack of an endemic essential oil bearing Asteraceae plant to use as a model, it is instructive to look at the studies that have been made of other essential oil plants. Examples have been taken from Australian endemic species, traditional essential oil crops and other newly investigated species from other countries.

II. LITERATURE REVIEW

ESSENTIAL OILS FROM THE AUSTRALIAN FLORA

In recent years an increasing amount of research has been undertaken into the essential oils of native Australian plants. By far the most productive genus is *Eucalyptus*. Three important species yield valuable oils for perfumery purposes. *Eucalyptus macarthuri, E. citriodora* and *E. staigeriana* yield oils with unique desirable characteristics.

It is known that a great many other Australian plants yield perfumes and oils of great fragrance. The flowers of many species have a special value in this regard, particularly some species of *Pittosporum* and *Hymenosporum flavum*. In particular, *Boronia megastigma* yields a scented oil of great delicacy and value.

Acacia farnesiana is the only Acacia so far used commercially for the production of scent and pomades, but there are many other species with similar possibilities. Species include *Prostanthera, Calytrix, Homalocalyx, Phebalium, Bosistoana, Baeckea, Melaleuca, Callistemon, Leptospermum,* and others (Althofer G.W., 1976).

Lassak and Southwell, (1980), in a short report, remind us that the Australian indigenous flora includes at least 4,000 species containing volatile essential oils in their leaves, wood, bark, fruits or flowers. A systematic survey of these oil-bearing species aims at the discovery of new essential oils with perfumery or flavouring potential and the identification of new sources of essential oils with established uses. For example, cineole-rich oils (used medicinally); piperitone-rich oils (a raw material for the manufacture of menthol); terpinene-4-ol rich oils (used both medicinally and as a flavouring agent).

So far, some 60 samples of foliage, belonging to species of *Eucalyptus, Melaleuca, Zieria, Eriostemon, Metrosideros, Senecio* and *Baeckea* have been distilled and characterised. Amongst these, *Melaleuca linophylla* from central Australia yielded 1.6 % oil, containing 52 % terpinene-4-ol, and shows potential as a commercial source of this bactericidal compound. Some Tasmanian and all King Island populations of *E. brookerana* yield substantial amounts of oil (ca. 2.5-3 %), rich in cineole (55-65 %), and may also have commercial potential.

Since this study is focussed upon one genus of the Asteraceae

family, the review which follows is confined to dealing with research that has concentrated on members of that family.

The Asteraceae (formerly Compositae) include many species of economic importance. These are *Aster, Helichrysum, Zinnia, Dahlia, Cosmos, Tagetes, Chrysanthemum, Anthemis* and *Cineraria*. In Tasmania, no endemic species are being used as a commercial oil producing crop. Of the Tasmanian species of Asteraceae, there are many which are odoriferous. Among them are: *Achillea millefolium, A. tanacetifolia, Anthemis tinctoria, A. cotula, A. arvensis, A. nobilis, Olearia argophylla, O. stellulata, O. phlogopappa, O. lirata, O. glandulosa, Graphalium luteo-album, Helichrysum paralium, H. ledifolium, H. purpurascens, H. ericeteum, H. argophyllum, Cassinia aculeata, C. spectabilis, Inula graveolens, Chrysanthemum leucanthemum, C. parthenium, Tanacetum vulgare, Senecio linearifolius* and *Bedfordia salicina*.

Tapping new sources of essential oil is always highly desirable, particularily with the increasing costs of synthetic materials and additives. This begins with the location of a suitable endemic clone, and bringing it into cultivation.

SCANNING PERENNIAL POPULATIONS

A survey of natural populations of promising essential oil species generally reveals that there is variation within the population. The necessity of maintaining uniformity of quality in an essential oil, requires that clonal material be selected and/or developed by plant breeding procedures.

The pattern of variation arises from a mixture of adaptations by genetic differentiation of morphological traits and phenotypic plasticity (Gray A.J., 1985). The plasticity, or developmental flexibility, can be regarded as a part of the genetic differentiation and an aspect of the 'adaptive armoury of plants' (Bradshaw A.D., 1973).

The majority of work which investigates variability of plants concentrates on tree species, which are not of the Asteraceae family. Despite this, the general principles that are brought out by these examples are still applicable here. For instance, the identification of clones is often sought by some morphological

yardstick. Often, however, there are no parameters that correlate well with those traits that are of interest.

The morphology of clones of the Para rubber tree, *Hevea brasiliensis* have been studied. The variation in regard to the density and size of ray groups, density of laticifers per row per unit circumference of the tree, diameter of laticifers and the extent of connections between laticifers were examined, along with yield. Although the extent of yield variability among clones was very high, morphological variations were not so marked. Clone identification based on gross morphological characters is not always reliable. However, the structural characters are more stable and reliable relative to environmental influence, while the bark anatomical parameters can be used for identification of clones (Premakumari D. *et al.*, 1985).

Another study of morphological and phenological variation has been made among five black cottonwood (*Populus trichocarpa* Torr. & Gray) clones from each of ten natural populations at one plantation site in western Washington. The 50 clones displayed a large range of variation in the 15 leaf, branch and phenological characters studied. Clones differed in 14 characters and populations differed in 10 of the characters charted. The results point to considerable genetic variation and great potential for clonal selection for black cottonwood improvement (Weber J.C. *et al.*, 1985).

An analysis of morphological variation in a field sample of *Caladenia catenata* (Smith) was made by Morrison and Weston. They found a great variation both within and between the populations studied, for all morphological attributes, and the ranges of the attributes overlapped considerably between sites. The results suggested that there were two phenotypically distinct polythetic taxa within the group. However, no exclusive or 'key' attribute was found to discriminate between them (Morrison D.A. and Weston P.H., 1985).

Variations in populations of plants may be extreme, in that each population is distinctly different from others. On the other hand, variations in characters from one population to the next may be so gradual that no definite lines can be drawn to separate one population from the next.

An example of one such population was demonstrated by a morphometric and chemical study of 24 populations of *Richea scoparia*

and *R. angustifolia* in Tasmania. The analyses failed to demonstrate any clear-cut discontinuities between the two species. The data are interpreted in terms of a cline of a highly variable species, with types varying in accordance with alpine climates from south-west to north-east Tasmania. Superimposed on this cline is a grouping into two sets related to geological substrates. These sets do not correspond to the taxonomic 'species' (Menadue Y. and Crowden R.K., 1983).

Much variation is attributable to localized environmental factors, such as is exhibited by natural populations of *Echium plantagineum* L. in south-eastern Australia (Wood H. and Degabriele R., 1985). Here, for instance, plants grown in drier conditions flowered later, had proportionally wider leaves and were smaller than well watered clones.

Variability in plants can also be expressed through differences in plant metabolites. The genetic and environmental variability of β-carotene and ascorbic acid in *Lycopersicon esculentum* Mill. was investigated by planting seven cultivars at different locations and dates. The characters tested were found to be significantly affected by both location and planting date. The β-carotene and ascorbic acid were heritable to an appreciable degree, and are suspected to be under multigenic control. The February planting gave higher β-carotene contents at one location than another, thus indicating a significant effect due to location. Also, the dry season planting gave higher values than the June planting in both locations, indicating that season has an effect on the β-carotene content. Ascorbic acid content is high in bright sunlight; therefore, the higher values of ascorbic acid obtained in February may be due to this factor. The variations in ascorbic acid are attributable to both genetic and environmental sources (Abani M.S.C. and Uzo J.O., 1985).

With a highly variable population of plants, there is always the possibility of locating a clone with exceptional characteristics. Superior genotypes have been identified in wild populations. For instance, *Vaccinium angustifolium* from northern and southern sites were collected and grown in a greenhouse environment. Genotypes from sites with low light levels exhibited greater numbers of inflorescence buds and flowers per bud than those from sites with high light levels. Genotypes from dry sites

produced larger fruit than those from wet sites. These data suggest that environments exist that favour natural selection of horticulturally desirable traits in this species (Pritts M.P. *et al.*, 1985).

Extensive investigations have been performed on the phyto-chemical variations between wild-growing and cultivated Argentinian aromatic plants. It was observed that specimens of the same botanical species growing in areas of close proximity could present striking differences in the chemical composition of their essential oils. For instance, the species *Lippia alba* is widely distributed in Argentina, and shows no morphological differences to admit a botanical variety. Oil from plants in one region are rich in lippione (1,2-epoxypulegone). Cultivated plants, on the other hand, contained only piperitone, which was attributed to artificial watering and the absence of calcium in the nutrition of these plants.

At the same time, plants from a second region contained neither lippione nor piperitone, but dihydrocarvone. It was thought that the calciferous waters of the area contributed to this phytochemical divergence. Also, differences in aroma characteristics were noted in populations of *L. alba* situated only short distances from one another (Retamar J.A., 1986).

This variation in natural populations has two major aspects. Firstly, it means that once a potentially useful plant is found, it is in the interests of effective quality control that oil is not derived from material harvested indiscriminantly. Rather, clonal selection is advisable. Secondly, on the positive side, a highly variable population implies that there is great scope for selecting a clone that possess all the desirable qualities necessary for the production of an acceptable essential oil.

NEW CROP ESTABLISHMENT

The introduction of a new species of essential oil plant into cultivation involves many facets. The first step is the selection of a suitable essential oil bearing species, usually on the bases of oil yield and organoleptic quality.

In the United States, about 500 plant species from various regions have been screened recently for their multipurpose, energy-producing potential. A recent report added 92 species to this list (Carr M.E., 1985). The author analysed the plant samples for 'oil', 'polyphenol', 'hydrocarbon' and protein. Among the plants studied, the oil yields ranged from 0.8%to 5.0% (dry, ash-free basis), however, the author does not indicate whether the oils are suitable for any commercial applications. In particular, many of the higher yielding species may have contained oils of use in industry as flavour, fragrance, or petroleum substitutes. This report appears to cover a vast range of species, at least on a superficial basis.

On recognition of a suitable species, a clone is selected from the population which is most appropriate for the purpose intended. Cloning is defined as the vegetative regeneration of a single genotype as represented by a single plant, single growing point, single meristem or single explant. Cloning is a powerful procedure both as a plant selection tool for breeding and as a plant propagation tool for reproduction. In nature, some species use vegetative multiplication as a major strategy for their adaptation. Such species reproduce by special vegetative structures such as root suckers, natural layering, rhizomes, bulbs and corms. Cultivation of various food and fibre crops (e.g. potato, yam, sugar cane, banana and bamboo) is based on development of clones and utilises such naturally occurring vegetative structures combined with division.

The discovery that certain woody trees, shrubs, and vines could be propagated by hardwood cuttings was the basis for the selection of clones of early horticultural crops of grape, fig and olive (Zohary D. and Spiegel-Roy P., 1975).

Vegetative propagation of species by means of cuttings is useful for clonal multiplication of desirable genotypes and for obtaining

genetically uniform material as planting stock. A cutting trial on poplar showed that the best growth occurred when the cuttings were not too small. That is, there is an optimum size of cutting below which performance was affected (Mathur R.S. *et al*., 1983).

The ability to propagate clones by leafy cuttings became possible with the development of propagation technology, such as greenhouses, mist facilities, and rooting hormones. The consequence of these developments has been to extend the cloning process to ever-widening lists of plants, particularly ornamentals.

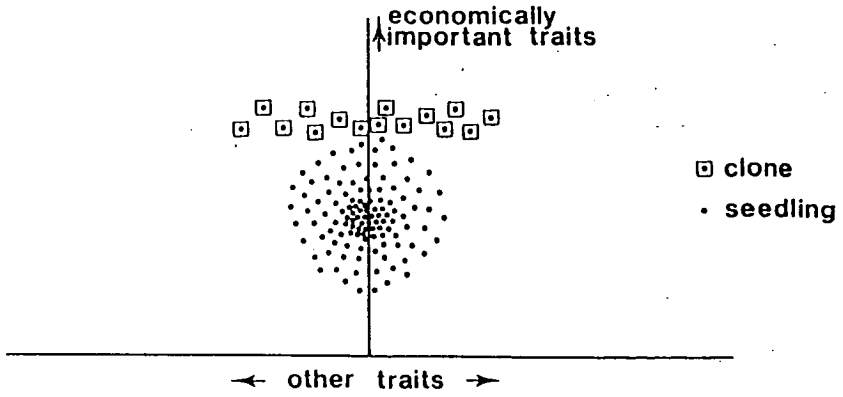
The benefits of cloning are as follows. Cloning provides an efficient means of :1) selecting specific genotypes of varying degrees of heterozygosity as cultivars, and 2) 'fixing' them immediately for subsequent propagation. The result is maximum phenotypic uniformity among 'superior' vegetatively propagated offspring and a powerful tool to standardise modern horticulture to the minutest detail both in product and production methods.

However, the fact that essentially all plants of a single clonal cultivar are identical genetically makes all plants equally susceptible to disease, environmental stress or some other factors.

An important factor in clonal propagation is variation potential that may result from inadvertant selection of the wrong clone, development of an inferior variant, or introduction of a systemic pathogen. The occurrence of any of these problems early in the multiplication process may result in production of thousands of copies before discovery.

Sources of variation within clones can be divided into four broad categories: 1. genetic mutations, 2. chimeral rearrangements of pre-existing mutants, 3. epigenetic changes and 4. systemic infection by pathogens. The occurrence of such variation can have either useful or detrimental effects (Kester D., 1983).

A selected clone might compare with a selected seedling population as shown in the graph below. The clones can be expected to out-perform the seedlings in economically important properties for which they were selected, such as growth rate, shape and resistances. Clones tend to show a wider range in other properties, for which perhaps no conscious selection was carried out, as the rare superior seedlings from which the successful clones were made, may represent extreme genotypes.



(from Heybroek H.M., 1984)

Low clonal heritabilities suggest lower genetic heritabilities, and thus great difficulty in a sexual improvement programme. Such is the case with *Dioscorea floribunda*. However, superior plants in such a programme should have great value as clones (Martin F.W. and Cabanillas E., 1967)

Clonal propagation is widely used in plant genetic engineering programmes. It is used as an alternative method for mass propagation of hybrid seed. It may also allow for the rapid development of improved plant varieties and replace the time-consuming conventional breeding procedures currently used for certain perennial varieties. In contrast to clonal propagation, which faithfully produces genetic carbon copies, regeneration of plants from callus, leaf tissue explants, or plant protoplasts (wall-less cells), using tissue culture results in recovery of somaclonal variants. Likewise, genetic variability occurs following the regeneration of plants from pollen or the male gametes in tissue culture (Sharp W.R. *et al.*, 1984).

A comparison of *Stevia rebaudiana* Beroni (Asteraceae) plants grown from seeds, cuttings and stem-tip cultures for growth and sweet diterpene glucosides gave the following results: There was no significant difference between the stem-tip cultured and seedling plants, both in growth and in chemical composition. The clonal plants showed significantly smaller variations in diterpene content than the sexually propagated plants; they were almost as homogeneous as the plants propagated by cuttings (Tamura Y. *et al.*, 1984).

The cultivation of novel, commercially viable plants, often requires their adaptation to different climatic conditions. Sometimes, a sub-alpine species will be grown at or near sea level,

or coastal plants may be taken inland. Therefore, it is desirable to select plants that exibit the necessary degree of frost (or perhaps drought) tolerance.

The mechanism of frost hardiness has been investigated in relation to seasonal variations of some compounds (water soluble and protein bound thiols, ascorbic acid, diverse sugars and amino acids). These are assumed to be involved in adaptation to freezing resistance. Spruce needles survive 0 °C all through the year. However, temperatures below zero degrees are resisted only through the winter months. The induction and maintenance of frost hardiness is a complex metabolic process. Absolute frost resistance is achieved if local dehydration can be avoided. Dehydration leads to deformation of cell organelles by intra-cellular ice formation.

In woody plants the cold-hardening process occurs in three stages:

1. Short day perception effect, which occurs in early Autumn (high day/low night temperature)
2. Physical and metabolic development, characterised by frosts, leaf fall and the beginning of dormancy
3. Dormancy during periods of permanent frost

(Grill D. *et al.*, 1987).

When temperatures remain far above 0 °C (September) the first step of the adaptation process occurs which results in an increase in osmotic pressure. The content of the water soluble thiols and ascorbic acid increases at the beginning of October whereas the sugars increase in November. Ascorbic acid prevents the denaturation of proteins and is an anti-oxidant for sulfhydryl components, which are themselves protective for proteins. However, the SH groups are protective only after the freezing point has been lowered by the increase in osmotic potential. This is confirmed by the fact that pollution stressed trees are high in SH groups, even at low osmotic levels, and are also sensitive to frosts.

Thus, during the acclimation process the osmotic pressure is raised by substances other than sugars. That is, by organic acids, glycosides and probably inorganic salts. In contrast to earlier work by the same author, the characteristic seasonal variation of the content of protein bound SH-groups was not observed. Furthermore, during autumn and winter, no accumulation of amino acids was detected. This was thought to be due to an unusually mild

winter.

At the end of winter, all recorded substances showed maximal concentrations. Amino acids, which remained at a constant level during the other seasons, reached a higher level at that time. With the spring flush of young needles, the substances lowered in concentration to typical summer levels (Weiser C.J., 1970). Frost tolerance of *Eucalyptus* leaves has been assessed by an electrical conductivity method, which was found to be a reliable indicator of leaf survival status. A significant difference in frost tolerance was found between unhardened and hardened seedlings (Raymond C.A. *et al.*, 1986), which points to the importance of careful nursery practices.

The water status of plants has a large bearing on their metabolic function, which may include the production of essential oil. Low leaf water potentials result in large reductions in photosynthesis. In higher plants, the reductions are caused both by decreases in the photosynthetic activity of a unit of leaf and in the production of new leaf surface. Photosynthetic activity declines because of decreased stomatal opening and the inhibition of chloroplast activity. Either may control photosynthesis depending on which is the more limiting at low leaf water potentials. The production of new leaf area is highly sensitive to water deficits and is usually reduced before photosynthetic activity decreases. This may be attributed to the high responsiveness of leaf enlargement to turgor. When low leaf water potentials are prolonged, leaf senescence often occurs and the quantity of existing leaf area may decline.

There is evidence that translocation is less sensitive to low leaf water potentials (Boyer J.S., 1976).

The satisfaction of a leaf's need for carbon dioxide requires an intensive gas exchange between mesophyll and atmosphere. The prevention of excessive water loss demands that gas exchange be kept low. Stomata open when a low carbon dioxide concentration in the guard cells triggers the uptake of potassium ions in exchange for hydrogen ions, the production of organic acids and the import of chloride ions, which induces hydropassive stomatal closure. That is, a loss of turgor occurs without a reduction of the solute content of the guard cell. It appears that these are insufficient to protect the plant from desiccation. An additional hydroactive

solute loss is necessary, and is brought about by (+)-abscisic acid (ABA) acting as a feedback messenger between mesophyll and epidermis (Raschke K., 1976).

Attempts to correlate values of stomatal conductance and leaf water potential with particular environmental variables in the field generally have only limited success, as they are simultaneously affected by a number of environmental variables. For example, correlations between leaf water potential and either flux of radiant energy or vapour pressure deficit show a diurnal hysteresis. If many values are plotted a scatter diagram results. However, a simple model may be adequate to relate leaf water potential to the flow of water through the plant.

The stomatal conductance of illuminated leaves is a function of current levels of temperature, vapour pressure deficit, leaf water potential (really turgor pressure) and ambient carbon dioxide concentration. Consequently, when conductance is plotted against any one of these variables, a scatter diagram is produced. Physiological knowledge of stomatal functioning is not adequate to provide a mechanistic model linking stomatal conductance to all these variables (Jarvis P.G., 1976).

Contradictory evidence exists which suggests that photosynthetic rate is unaffected by induced water stress. For instance, *Parthenium argentatum* Gray. was grown in well-watered and water-stressed conditions (70 days: available soil water declined from 100% to 0%). Measurements indicated no significant difference in net photosynthesis between the wet and dry plots (Allen S.G. and Nakayama F.S., 1988).

The importance of determining plant water status in horticultural water requirement research is well recognised. Unfortunately, the choice of measurement is often determined more by convenience or availability of an instrument or technique rather than direct relevance to the aspect of plant water relations under study (Spomer A.L., 1985).

Many methods of gauging the water status of plants exist including measurements of evaporation, leaf conductance, leaf water potential (turgor pressure) and osmotic potential. The uses and limitations of the pressure chamber has been reviewed (Turner N.C., (1987). The pressure chamber has become widely used in the studies of plant water relations because of its relative ease of operation

and versatility. It has wide appeal especially for field measurements of total water potential. It must be recognised, however, that information on tissue water relations may have little or no relation to plant behaviour under drought. For instance, wild and cultivated sunflower that differed widely in their drought resistance had similar tissue water relation characteristics (Sobrado M.A. and Turner N.C., 1983).

Alternative measurement systems have included equilibration with microdroplets of polyethylene glycol 8000 (Fisher D.B., 1985) and micrometer leaf thickness measurements (Burquez A., 1987).

Measuring the stomatal conductances of potentially transpiring plants using porometry is seen by some as lacking relation between the measurements taken and the actual water status of the plant. It seems that the very act of stomatal conductance measurement alters a potentially transpiring plant's evaporative water loss rate. Under very high vapor pressure deficit (VPD) conditions, the directly measured conductance value (although correct for the leaf in the chamber) may be much reduced from that characteristic of comparable non-chamber-encumbered plants in the free air (Idso S.B., *et al*, 1988).

Leaf diffusive resistance has been found to have a tendency to increase with declining soil moisture content (Richards R.A. and Thurling N., 1978), and was greater at the adaxial leaf surface of rain-fed rape plants than in irrigated pots until moisture stress built up in the field plots. In addition, the adaxial and abaxial surfaces showed similar trends in diffusive resistance variations (Clarke J.M. and McCraig T.N., 1982).

Other work has concentrated on developing a simplified plant stomatal resistance model and validating it for potentially transpiring and water-stressed plants. The model requires the input of four parameters: canopy aerodynamic resistance, upper-canopy foliage temperature, air vapour pressure deficit and temperature (Idso S.B., 1988).

Two primary methods have been used to induce water stress in plants - withholding water and using osmotic agents. Water withholding treatments are more natural but are difficult to quantify unless pre-selected criteria for end points are established. The recent development of a system for imposing water stress by controlling the water column height and/or hydraulic

conductivity is currently the most promising method to date for inducing water stress on a long-term basis (8 weeks or longer) (Snow M.D. and Tingey D.T., 1985).

Osmotic agents, for instance sodium chloride (NaCl), polyethylene glycol (PEG), mannitol, sorbitol, and dextran have the advantage of inducing more rapid and precise levels of osmotic stress than other techniques but often induce toxic side-effects. Although PEG treatment has been assumed to be synonymous with water stress treatment, the effects of osmotically induced water stress may be quite different from those of water stress alone (Krizek D.T. 1985). The use of a membrane system to separate the osmotic agent from the root system eliminates many of the undesirable features of using PEG, mannitol and so forth (Tingey D.T. and Stockwell C., 1977).

The success of establishment of a new crop is, dependent upon whether it is suitably adapted to grow in the chosen environment. In some circumstances, it is necessary that some degree of frost tolerance and/or drought resistance is shown. The actual physical layout of a field of the new plant will to some extent determine the capacity of the plants to give maximum yields. Some plants are sensitive to competition, while others respond to the added pressure by producing more harvestable material.

COMPETITION

One aspect of competition is the relationship between plant yield and population density. There is now a general acceptance of the so-called inverse relationship. Here per-plant yield, ω, is related to density, D, as,

$$1/\omega^{\Theta} = A + BD \qquad (1)$$

where A, B and Θ are constants.

This form of the equation has been questioned with respect to the interpretation of the parameters. Recasting the original equation (1) in a slightly modified form, it has been suggested that the traditional formulation implies an assumption of equal competition within a specified region. Using experimental data from tomato and bean beehive experiments, this assumption has been rejected and an alternative form of the equation has been proposed (2),

$$\omega = \frac{W_m}{1 + qD^b} \qquad (2)$$

where W_m is the biomass of the plant without competition, q is a measure of competition including its intensity and the area within which it operates and b is a measure of the rate at which competition decays as a function of distance between plants (Vandermeer J., 1984).

One of the most important density dependent effects is competition between individuals of the same species. The effective density experienced by an individual depends on the number, size and position of neighbouring individuals. Several outcomes may be seen: (a) Where competition results in plants of different sizes, plant size and distances among individuals will be positively correlated. (b) Where competition results in mortality, either the overall spatial pattern will shift to a more regular distribution or there will be fewer individuals than expected by chance in the immediate proximity of other individuals.

The effects of spacing may be confounded with microenvironmental differences. Thus, plants growing in poor conditions may be small, have a low survival rate and be far apart; while those growing in good conditions may be close together, but

large and with a high survival rate.

In experiments where plants are grown over a range of densities, three or perhaps four phases in the response of the population to density can be recognised. These phases also reflect a time course of behaviour of individuals within a single pure stand as they increase in size from seedlings to adults.

How the presence of another species influences population regulation is frequently misunderstood. Inter-specific competition is often quite erroneously equated with a density-dependent effect (Antonovics J. and Levin D.A. 1980).

Experiments on the effects of plant spacing and season of growth on *Melaleuca alternifolia* were performed by Small. He compared the response to three within-row spacings and measured the effects of seasons over several harvests. He found that there was an average increase in leaf and oil yield of 93 % (2.9 t/ha and 46 l/ha, respectively) in the highest population (26,908 trees/ha), compared with the lowest (6,727 trees/ha). He concluded that this native is amenable to cultivation for tea tree oil production, with plant spacing being an important factor in management. The optimum population exceeds 27,000 trees per hectare (Small B.E.J., 1981).

Intense competition is often present among natural stands of native shrubs and trees. Release from competition results in significant developmental responses. Most work in this field has, understandably, been done with trees in plantation situations. The significant relationships between basal area index in *Eucalyptus sideroxylon* and initial diameter of regrowth stems indicates that even very small stems growing in extremely dense regrowth clumps have the capacity to respond to release (Kellas J.D. *et al.*, 1982). This is in agreement with Squire R.O. and Edgar J.G., 1975.

Similarily with Jarrah (*Eucalyptus marginata*), thinning of regrowth involving up to 50% reduction in basal area, results in remarkable increases in diameter growth. More severe thinnings, while further increasing diameter increment of individual trees, lead to a decline in basal area increment of the stand. Crown vigour and position relative to the forest canopy are good predictors of diameter growth rates (Abbott I. and Loneragan O., 1983).

Thinning, or pruning, of crop plants often takes place on a regular basis. Plants may be pruned to conserve or alter the shape

of the canopy, or for the collection of commercially valuable material. The severity, shape and timing of the pruning profile can largely determine the resulting yield and future growth potential of individual plants.

Oil yield of *Mentha piperita* L. is influenced by harvest date and plant density. Yield is maximal early in the growing season, with plant densities of 30 or 40 plants/m^2. With 10 plants/m^2, yield continues to increase even at a menthol content of 50%. At the higher densities, the herb harvested when oil contains 45% of free menthol, resulted in maximum oil yield and optimal oil quality. If harvest is delayed, the menthol content increases, but at the expense of increased levels of menthofuran and decreased oil yield. As the growing season progresses, menthol and menthyl acetate contents increase, while menthone decreases. This effect is accelerated by high plant densities (Clark R.J. and Menary R.C., 1979).

It has been observed that, like other tree plants, commercial tea bushes partition dry matter in a similar way. That is, the leaf mass as a proportion of the total dry matter decreases with age while that of woody parts increases. Consequently the yield proportion (harvest index) of the total dry matter is smaller in old tea bushes than in young ones.

The effect of pruning at different heights has been investigated for *Camellia sinensis*. Results show that yield of leaves per plucking area were greatest in the smallest bushes (Magambo M.J.S and Waithaka K., 1985).

Tarragon (*Artemisia dracunculus* L.) is a perennial member of the Asteraceae, which is cut to very short stumps at harvest time. In fact, the crop management of this herb allows for two harvests to take place each year. The first harvest takes place in the middle of summer (July), after which the plants are able to put on enough growth to make a second harvest possible in September (Guenther E., 1949).

The only other member of the Asteraceae used for commercial essential oil production using leaf material is Wormwood (*Artemisia absinthium* L.). This crop is also harvested late in July (Northern Hemisphere), when the shrubs are about a metre high, and in full bloom. Delayed harvesting has been found to decrease oil yield and quality. The harvested stems are tied into sheaves and dried for 24

hours before distillation to facilitate the removal of oil from the foliage. It has been observed that distillation of fresh herb results in markedly poorer yield (Guenther E., 1949).

Thus, the outcome of any oil production venture is heavily dependent on crop management procedures. The initial setting up of the plot, including spacing; the irrigation schedule; the pruning and/or harvesting severity, timing and style, will all be reflected in the final yield and quality of the essential oil. In addition, factors such as pre-distillation drying of the herb and distillation time are also yield determinant.

Of these, one of the most important factors is the timing of harvest. This necessitates a familiarity with the responses of the plant to changing environmental conditions throughout the year.

SEASONAL VARIATION

The way in which the essential oil content varies in oil-bearing plants throughout the year is of interest from an economic point of view as well as a purely acedemic one. The optimum time of harvest, with respect to both oil quantity and quality, must be considered to obtain to best possible oil. A mistake in judgement as to the most suitable time for harvest could easily result in a reduced yield of poor quality oil.

Work on *Eucalyptus camaldulensis* and *E. polyanthemos* Schauer. showed the following. Fresh adult leaves yielded volatile oil, the amount of which decreased significantly throughout the winter season. During spring a slight increase was observed, followed by a steady rise in essential oil content through the summer months. The cineole content of the two species was not significantly affected throughout the year. In addition, the seasonal cycle of rutin content in the leaves of the two species showed a significant increase in the hot months. The drop in rutin content in winter may be due to low temperature, the short day and the low light intensity. The data showed that *E. camaldulensis* has higher levels of volatile oil with a high cineole percent and a low rutin content. Conversely, *E. polyanthemos* has less oil with a low cineole content and a high rutin content (Abou-Dahab A.M. and Abou-Zeid E.N., 1973).

The way in which plants partition the essential oil produced between juvenile and mature leaves also affects management decisions. In *Artemisia dracunculus* the young leaves contain a higher yield of total oil than mature leaves. This is in contrast to many essential oil crops, where the older leaves are the major contributors to oil yield, as in peppermint, for instance.[1] However, in *Artemisia* sp. the older leaves had a higher content of methylchavicol. In addition the amount of oil rises from the beginning of the vegetative period to the beginning of bud development, then decreases somewhat, reaching its maximum during flowering time then declining to leaf fall. Conversely, in a study of *A. douglasiana*, no consistent diurnal pattern was observed, yet there was evidence of changes in the total volume of oil without changes in the composition (Scora R.W. *et al.*, 1984).

[1] (Clark R.J. and Menary R.C., 1984).

A monthly variation of oil yield and composition has been described in *Salvia officinalis* leaf oil. The maximum yield occurred in July (Northern hemisphere), and it is recommended that plants are harvested in October to maximise the percentage of thujone (Pitarevic I. *et al.*, 1984).

The way in which some essential oil crops vary in their oil content and quality can be quite complex. Peppermint (*Mentha piperita* L.) oil has been thoroughly investigated and may serve as an example. An oil is produced that 'matures' over the growing season. This involves increasing menthol and menthyl acetate concentrations, while menthone levels decrease. The Tasmanian oils are characterized by low limonene and menthyl acetate levels, and to a lesser extent, high menthone, menthol and low cineole contents, relative to most major production areas. The highest menthyl acetate and menthol concentrations are associated with the onset of flowering. In regrowth herbs, flowering does not occur, and the observed maturation of the oil may be the result of cooler nights at that time, or cessation of growth due to the onset of dormancy (Clark R.J. and Menary R.C., 1981).

The effect of photoperiod has also been investigated in relation to yield and composition of peppermint oil. Cold nights and long photoperiods are seen to lead to increased menthone and menthol, with decreased menthofuran and pulegone concentrations. Photoperiod itself is an important determinant of monoterpene composition, rather than photosynthetic factors (Clark R.J. and Menary R.C., 1979a).

Essential oils which are produced by plant structures are not confined to the plant. Rather they are readily emitted into the atmosphere in variable amounts. The rate and seasonal variation of these emissions has been investigated in a pine forest. Emissions were high in summer and low in winter, with emissions being dependent on temperature. The monoterpene emission rate is influenced by light, resulting a lower rate in winter than predicted by purely a temperature difference. A total monoterpene emission rate was calculated to be 0.32μg/g(dry wt.)/hr (Yokouchi Y. *et al.*, 1984).

In contrast to many of the higher plants containing essential oil, there are some plants whose percentage oil content does not change during the year. *Bazzania trilobata* grows in large dark

green mats on the forest floor. The oil contains α-gymnomitrene, β-gymnomitrene, β-bazzanene, calamenene, β-chamigrene, cuparene, drimenol, 5-hydroxycalamenene and isobazzanene. The percentage composition of its oil was stable throughout the year (Huneck S. *et al.*, 1984).

Once the potential of a particular plant as a source of essential has been realised, the process of determining the optimum time for harvesting is vital. Such information is the basis of sound horticultural management of the crop.

ESSENTIAL OIL QUALITY

The primary uses of essential oils bring them into situations ranging from perfumes to household cleaners. In all instances, the usefulness, quality and acceptability of these products are determined by the human sense of smell.

Currently there are some five theories which attempt to explain the mechanism of olfaction, however, this receptor system still remains largely unidentified, and there is no odour theory that can really explain the actual process of odour perception (Grub H., 1980; Brud W.S. 1980):

1. Adsorption/Desorption.
   Odorants stay fixed on the olfactory membrane for only a short time, during which some of their molecules penetrate the membrane. So, characteristic odour is directly related to the intensity of this interaction, and thus, molecular size (penetration hypothesis). Adsorption of the odorant triggers electrical phenomena (Dravnieks A. *et al* ., 1979). The molecules of odorants are electrically attracted by the membrane, arranging themselves on the receptor cell in accordance with the distribution of electrical charges in the molecule (Steiner W. in Sturm W., 1978).
2. Enzyme hypothesis.
   Olfactory stimuli result from a reaction between odorants and enzymes of the olfactory receptors. Numerous metabolic processes involving specific proteins take place in cells, but only in the presence of certain chemical compounds. Additionally, the proteins and enzymes of the olfactory membrane have been shown to behave in this way (Kistiakowsky

G.B. in Roderick W.R., 1966).

3. Oscillation hypothesis.

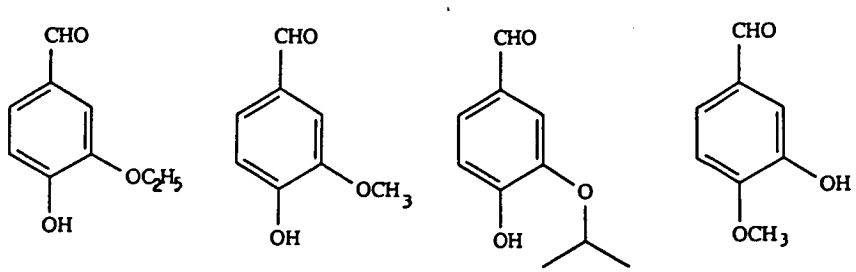
   This is based on the supposition that characteristic molecular vibrations are responsible for the interaction with receptors. Work has shown that the entire spectra of molecular vibrations penetrates the membrane, while the molecules themselves do not (Davies J.T. in Roderick W.R. 1966).

4. Stereochemical hypothesis.

   Since contact between the odorant and the olfactory membrane is a prerequisite for perception, molecular shape is likely to be a factor. Odours have been classified into seven main groups, the hypothesis being founded on the close relationship between external shape of the molecules and the olfactory impressions to which they give rise. Hollows are postulated on the olfactory membrane into which molecules of aroma chemicals fit like a key into a lock. In these terms the absence of specific hollows offers an explanation for anspira (Amoore J.E., 1970). This theory successfullly accounts for the identical odours of isotopic molecules and the different odours of stereoisomers; the two chief contradictions in other theories.

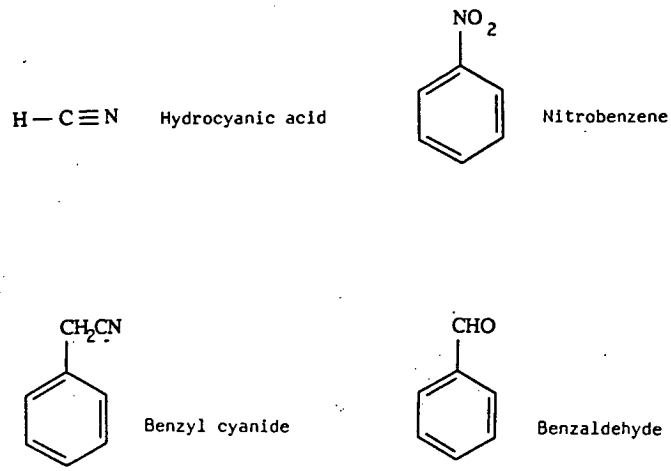
5. Functional group profiles.

   This theory, proposed by Beets M.G.J. (1978), is based on the spatial position of functional groups and their charges, which determines the dipole moment of the molecule. Fixation of molecules on the surface of the receptors is achieved by the functional groups, whereas the stimulant effect is brought about by the profile of the molecule and the orientation on the receptor. Molecules of similar structure should have similar smells:



Ethyl Vanillin	Vanillin	Iso propyl Vanillin	Iso Vanillin
VERY STRONG	STRONG	WEAK	VERY WEAK

An olfactory impression is caused only by the molecules that come into contact with the olfactory membranes. There is a relationship between molecular shape and sensory impression - this has been confirmed by experimentation. Different substances create different odours, or, on the other hand, cause similar or identical odours e.g. substances which have the odour of bitter almonds:



There is a belief that certain functional groups impart characteristic odours whenever they occur. This seems to be generally true, but it has been also noted that bulky hydrocarbon groups near the functional group can weaken or obliterate the odour quality. Aliphatic amines and sulphur compounds are very resistant to steric hinderance, yet they are greatly weakened by electron withdrawing groups. Aliphatic compounds having a multiplicity of methyl groups have the odour of camphor or menthol (Brower K.R. and Schafer R., 1975).

By contrast, similar compounds can have different smells. Contact between olfactoric and protein molecules of the olfactory membrane receptors is physical in nature. Specificity of receptors is observed since some people cannot perceive certain odorants, as in partial anosmia.

Olfactory fatigue results from the collapse of the electrical potential at the extremities of the network due to the bridging of too many active nodes. The fact that some substances are odourless can be interpreted in two ways. Firstly, their molecules might be too large to penentrate into the network and thus to modify the energy state. Alternatively, there may be no pattern of active

nodes in the protein net which fits the arrangement of active groups of the molecule.

Application of physical and chemical specifications alone is not sufficient for determining either quality or acceptability of an essential oil. For instance, samples of Lavandin Abrialis with a camphor content exceeding 10% often fall within the Essential Oils Association limits but rate poorly organoleptically. Also, samples of a high olfactory quality may fall outside these limits (Garlick B.K., 1977).

Four chemotypes of *Origanum vulgare* L. have been examined with respect to the yield and quality of essential oil, and the types and distrubution of their glandular hairs. The types varied in phenol, thymol and carvacrol content in their essential oils. While there were no differences in structure of the peltate and capitate hairs between chemotypes, the density of the peltate hairs varied and appeared to be correlated to the total essential oil content (Werker K. *et al.*, 1985).

Secretory tissues can be divided into two main types, based on their origin and location:

1. Secretory tissues which occur on plant surfaces (e.g. glandular trichomes) and usually exude the secreted substances directly to the outside of the plant (exogenous secretion).
2. Secretory tissues which occur inside the plant body and secrete into specialised intercellular spaces (endogenous secretion). The intercellular spaces may develop schizogenously or lisigenously or by a combination of the two. In some cases, as for instance in the laticifers, the secreted material is accumulated inside the cells.

The glandular trichomes are constant features characteristic of many plant species and develop without external stimuli. The question whether external factors may influence their density on the plant surfaces has recently been studied (Fahn A., 1988).

The inner secretory tissues, such as ducts and cavities, are also characteristic of certain plants, but their development may or may not depend on external factors, such as injuries and pathogens, or on physiological stresses. Ethylene appears to be the most important factor in the formation of resin and gum ducts. In the epithelial duct cell the resin is synthesised mainly in the

plastids, although other cell organelles may also be involved in this process. The gum is produced by the Golgi apparatus.

It is suggested that there is an evolutionary trend in the development of the secretory tissues from scattered cells to organized ducts and cavities, culminating in glandular trichomes (Fahn A., 1988).

With regard to terpenoid secreting tissues, a classification according to their progressive evolution has been put forward. Four types are recognised:

I Endogenous secretory tissues with intra-cellular accumulation of secreted material.

II Schizogenous endogenous secretory tissues with extra-cellular accumulation of secreted material

III Secretory tissues with schizo-lysigenous lumen and

IV Exogenous glandular structures (glandular trichomes) (Denissova G.A., 1975).

Members of the Asteraceae have been observed to carry peltate hairs, where material is secreted between the adjoining walls of the head cell (Werker E. *et al.*, 1985). The leaf trichomes of some *Parthenium* species have been studied. Each has a charcteristic combination of leaf trichomes. All species had two or more types of trichomes, and in some species the trichomes on the upper and lower epidermal surfaces were different. The prominent trichomes on the upper epidermis of *P. tomentosum* and *P. fruticosum* were simple, uniseriate and conical. These were also observed on both epidermal surfaces of *P. shottii*. Extremely long, narrow, simple, whip-like trichomes formed a dense cover on both surfaces of *P. rollinsianum* and on the lower surfaces of *P. tomentosum* and *P. fruticosum*. Simple, uniseriate, cylindrical trichomes, and biseriate, glandular trichomes were observed in all four species (Healey P.L. *et al.*, 1986).

The physiological process of oil synthesis culminates in the production of an extractable oil. This oil is perceived by the human olfactory system which is the most critical of evaluatory devices. The quality of an oil is thus determined in an very subjective fashion, since the oil will be either acceptable or not, irrespective of the judgements given by electronic means of analysis. However, analysis of essential oils plays a significant role in the determination and characterisation of oils today.

ANALYTICAL TECHNIQUES

The basic aim of flavour and fragrance analysis is the identification of the important organoleptic components found in essential oils or finished products. In pursuit of this aim researchers have developed different techniques for determining which compounds are of interest organoleptically, as well as for the identification of these compounds. Gas chromatography (gc) and, in particular, capillary-column gas chromatography have been found to be of great utility in this field.

The important organoleptic components in a fragrance mixture are primarily chemicals of high volatility. This is one of the main reasons why gas chromatography has played such an important role in the analysis of fragrances. A second important feature is that fragrances are generally quite complex in composition. It is not uncommon to find that an essential oil contains hundreds of individual chemicals that interact together to give the overall character of the oils' fragrance.

The fundamental problems encountered in the analysis of the oil are: firstly, to obtain the resolution necessary to distinguish each chemical; secondly, to obtain the necessary sensitivity in order to detect all components; thirdly, to determine which components are the most important to the overall fragrance; and finally, to identify all components.

Vapour-phase gas chromatography can be used to determine the terpene content of solvent extracted essential oils. The amount of terpenes found in the oil can vary with the method of extraction. For instance, with steam distillation a lower proportion of α-pinene was evident, perhaps due to a certain amount of oil remaining associated with the leaves which cannot be removed. Furthermore, structural rearrangements could be occurring in heat-labile compounds. In addition, when extracting with solvent, the temperature is more important in bringing about total extraction than the length of time the material is in contact with the solvent (Ammon D.G. *et al.*, 1985).

An aid to the separation of essential oil components for gc analysis has been reported. A modified dry column chromatography procedure makes possible the rapid separation of complex mixtures

into five fractions of different polarity. Fraction 1 is obtained by elution of the samples on a silica gel column with pentane. The second is derived from elution with benzol, and the remaining polar parts are divided into three portions and eluted with ether/methanol (8:2, v/v). Since the individual fractions contain less components than the total sample, a better gas chromatographic separation is achieved. This is of particular importance when packed columns are used for subsequent identification by mass spectroscopy, nuclear magnetic resonance and infra-red spectroscopic procedures (Kubeczka K.-H., 1984).

Gas chromatography exit-port organoleptic evaluation has become an important method for determining which of the many chemicals present in a mixture are actually responsible for the character of the oil (Motto M.G., 1987). Gas chromatography/mass spectroscopy headspace analysis techniques enables the investigation of volatiles from whole flowers, leaves, fruits and so on, as well as from essential oils. The flowers from two *Rosa* species were shown to have distinct chemical profiles composed of aliphatics, terpenoids, and aromatics. This method detected only one-third of the identified components (Dobson H.E.M. *et al.*, 1987).

Capillary gc-Fourier transform infra-red spectroscopy (gc-Ftir) is a relatively new technique, and only recently has it been applied to essential oils and related samples. The amount of structural information which can be obtained from the infra-red spectrum of an organic compound is generally quite high. Compound class distinction, isomer differentiation, and often an exact identification can be made on the basis of the infra-red spectrum. Fine structural differences can be picked up, such as between the terpenes bergamotene and α-bisabolene. In addition, information can often be obtained from infra-red spectroscopy which may be difficult or impossible to obtain by other techniques. For example, some functional group determinations and the assignment of isomers is impossible to determine from mass spectrometric data, since even quite different compounds may yield identical mass spectra (Smith S.L., 1987).

It has been well documented that gc-Ftir and gc-ms provide complimentary structural information. As has been demonstrated in environmental, polymer and biological studies, the additional information from the infra-red spectrum is often invaluable in

making an exact identification (Smith S.L., 1987).

A liquid-solid column chromatographic method (lsc) using a silica gel column and applying a 2.5%-50% gradient elution of ethyl ether in pentane has been used for the analysis of naturally occurring oxygen-containing monoterpenes. This prefractionation led to better gas chromatographic separation and identification. The elution sequence during LSC gave extra information about the functional group of the compounds. Isomerization reactions are avoided by using purified and deactivated silica gel (Scheffer J.J.C. *et al.*, 1977).

High performance liquid chromatography (hplc) is also well suited for the separation of essential oil components and sometimes shows significant advantages over currently applied thin layer (tlc), and preparative gas chromatography methods. Since analysis is generally performed without exposure to air as in the case of tlc, and at ambient temperature, the destructive temperatures needed in gas chromatography are avoided and degradation products are less likely to be encountered (Kubeczka K.H., 1973).

A reversed phase hplc procedure for the separation of essential oils applies a water-acetonitrile elution system on octyl and octadecylsilane-bonded silica. Complex mixtures of sesquiterpenes and oxygenated volatile constituents can be resolved, with quality comparable to that of gc analysis. Photometric detection at different wavelengths can be an important parameter in optimizing the separation of essential oil constituents (Strack D. *et al.*, 1980).

Among the factors that are responsible for the separation observed on reversed phase columns is adsorption on residual silanol groups. Others include reversed phase interactions and partition chromatography between the mobile phase and the adsorbed layers of solvent (Hancock W.S. and Sparrow J.T., 1984). The distinction of two elution peaks requires a certain minimum resolution R (1,2). This depends on the separation system and can be described by the following formula:

$$R(1,2) = \frac{N}{4}\,\frac{\alpha - 1}{\alpha}\,\frac{k'}{1 - k'}$$

for $k'_1 = k'_2$

where $\alpha = \dfrac{t'_{R2}}{t'_{R1}}$

$k = \dfrac{t_R - t_M}{t_M}$

t_M = dead time

t_R = retention time

N = number of theoretical plates

(Engelhardt H., 1986)

Nuclear magnetic resonance (nmr) has recently become a useful tool for the study of plant extracts. In particular, carbon-13 nmr has been used for determining the presence of lignins and cellulose as well as non-structural carbohydrates (Maciel G.E. *et al.*, 1985 and Lapierre C. and Monties B., 1984).

A major study of nearly 100 monoterpenes by C^{13} nmr was carried out in 1975. This method proved useful in characterising these compounds, especially in the elucidation of stereochemical isomers (Bohlmann F., *et al.*, 1975).

Even such measurements as dry matter determinations have come under scrutiny to try to improve the reliability of the method. A novel technique uses near infra-red spectrophotometry for the non-destructive analysis of agricultural products to determine the percentage dry matter (% DM). Transmittance data is recorded at 906nm, which is in the vicinity of an absorption band associated with carbohydrates. The conclusion was drawn that there is a direct association between the regression equation that was developed to predict dry matter of onions and the concentration of carbohydrates in the onions, the major constituent of the dry matter (Birth G.S. *et al.*, 1985).

The advent of new techniques and the further application of established ones has meant that a plethora of new compounds have been identified in essential oils. It has also led to the more complete characterisation of oils, and an augmented understanding of the components they contain which are responsible for the individual character of each oil.

CHEMISTRY OF ESSENTIAL OILS

A simple definition of 'essential oil' is that it is a mixture of volatile products of plant metabolism which are deposited in special oil cells or oil receptacles and are sources of odour and flavour. Volatile essential oil components comprise mainly monoterpenes and sesquiterpenes. The monoterpenes may by catalogued conveniently as acyclic, monocyclic and bicyclic. Table II.1 lists some important members of each category, subdivided into hydrocarbons, alcohols, aldehydes, ketones and esters.

TABLE II.1

MONOTERPENES STRUCTURAL CATEGORIES

	ACYCLIC	MONOCYCLIC	BICYCLIC
HYDROCARBONS	ocimene	limonene	sabinene
	myrcene	phellandrene	thujene
		terpinenes	pinenes
		terpinolene	carenes
		p-cymene	
ALCOHOLS	nerol	carveol	sabinol
	geraniol	menthol	thujyl alcohol
	citronellol	piperitol	myrtenol
	linalool	isopulegol	fenchyl alcohol
		terpineol	borneol
		carvacrol	
		thymol	
		pulgeol	
ALDEHYDES	citral	perillaldehyde	
	citronellal	phellandral	
KETONES		carvone	umbellulone
		dihydrocarvone	thujone
		piperitone	fenchone
		carvomenthone	camphor
		carvotanacetone	
		pulegone	
		isopulegone	
ESTERS	linalyl acetate	terpinyl acetate	bornyl acetate

(Erickson R.E., 1976)

In the literature, no reference was found concerning the function and metabolism of essential oils in plants of the Asteraceae. However, in peppermint, monoterpenes which accumulate in mature leaves of flowering peppermint plants undergo metabolic turnover by a mechanism involving the reduction of *l*-menthone to the epimeric alcohols *l*-menthol and *d*-neomenthol. The latter is

preferentially converted to the β-D-glucoside and transported to the rhizome. Here the glucoside undergoes hydrolysis and oxidation back to menthone, then to *l*-3,4-menthone lactone. There is some evidence that suggests that the terpene is degraded to acetyl-CoA and reduced pyridine nucleotide, either of which may be subsequently used in the biosynthesis of other isoprenoid lipids as well as acyl lipids of the rhizome. The metabolic turnover of monoterpenes represents a mechanism by which a carbon and energy supply of the leaves (as accumulated monoterpene) is recycled in the developing rhizome (Croteau R. and Sood K., 1985).

While the major constituents of essential oils are generally mono-and sesquiterpenes, in certain plant families or genera other chemical classes contribute to the compostion of the oil. Phenylpropanoid compounds are among those found, sometimes as the main components.

It is well known, however, that lipid soluble, non-volatile components can be accumulated in essential oils and that odoriferous and flavouring substances can be produced by plants which do not possess special accumulating structures; these latter plants are therefore not counted among the essential oil plants in a strict sense. In such cases, the sources of odour or flavour originate from catabolic turnover of previously water-soluble and non-volatile precursors. For example the phenyl propanes often occur originally as glycosylated phenols and it is only after hydrolysis that volatile compounds appear. Similar examples are being unearthed where monoterpene glycosides are found in a water-soluble and non-volatile form outside the accumulation organs for essential oils. Some compounds originating from such bound forms (e.g. coumarin and vanillin from the phenylpropane group) are of great importance. The coumarins are a group of compounds arising from cinnamic acid or from hydroxycinnamic acids. They occur in their native state virtually exclusively as glycosides and they appear as components of essential oils in most cases after being set free from their glycosides by hydrolytic cleavage. Only coumarin itself and its simple derivatives are sources of odours and flavours. They are found in the Asteraceae and seven other families (Freidrich H., 1976)

The production of a particular odour may be dependent upon the presence of an extremely complex mixture of chemical components.

For instance, the tomato aroma has been found to contain the following 47 constituents. Some of these, such as i-butylthiazole and ionone compounds make a greater contribution to the overall odour impact than many of the major components.

acetaldehyde
acetic acid
propionic acid
γ-butyrolactone
butan-2-ol-3-one
i-valeraldehyde
pent-4-ene-3-ol
i-valeric acid
2-methylbutyric acid
2-methylbutan-1-ol
phenol
trans,trans-hexa-2,4-dienal
trans-hex-2-enal
cis-hex-3enal
γ-caprolactone
hexanal
cis-hex-3-ene-1-ol
hexan-1-ol
benzaldehyde
salicylicaldehyde
benzyl alchohol
o-cresol
guaiacol
trans,cis-hepta-2,4-dienal
trans,trans-hepta-2,4-dienal
2-i-butylthiazole
phenylacetonitrile
phenylacetaldehyde
o-hydroxy-acetophenone
methyl salicylate
2-phenylethanol
p-ethylphenol
trans,6-methylhepta-3,5-diene-2-one
6-methylhept-5-ene-2-one
γ-octalactone
caprylic acid
p-vinylguaiacol
γ-nonalactone
2,6,6-trimethyl-2-hydroxycyclohexanone
eugenol
trans,trans-deca-2,4-dienal
linalool
2,6,6-trimethyl-2-hydroxy-
cyclohexylidene acetic acid lactone
β-ionone
5,6-epoxyionone
geranylacetone

(Viani R. *et al.*, 1969).

ESSENTIAL OILS FROM ASTERACEAE (COMPOSITAE) SPECIES

The essential oils of *Artemisia* species have often been the subjects of investigation. *A. iwoyomogi* contains the monoterpenes α-thujone, camphor, borneol and piperitone as well as the sesquiterpenes germacrene-D, bicyclogermacrene, spathulenol and the eudesmanolide isotelekin. *A. gmelinii* oil contains five guaianolides in addition to α-thujone, camphor, germacrene-D, bicyclogermacrene and α-humulene (Greger H. *et al* ., 1986). The aerial parts of *Artemisia anomala* S.Moore afforded, in addition to umbelliferone, herniarin and salvigenin, the eudesmanolides reynosin and armexifolin, the guaianolides dehydromatricarin and its desacetyl derivative, two secoguaianolides, a dimeric guaianolide and two derivatives of phenylalanine. This plant has been used for centuries by the Chinese for its function as an analgesic, haemostatic, antibiotic and for curing wounds (Jakupovic J. *et al* ., 1987).

Four Brazilian Asteraceae have been investigated on the basis of their popular usage. *Bidens bipinnata* and *Pectis apodocephala*

Baker are used as a diuretic and as a sedative respectively. In addition, *B. bipinnata* is used in China against rheumatism, weakness and furunculosis, showing also anti-cancer and anti-inflammatory activities. These two species, together with *Verbesina diversifolia* and *Wedelia scaberrima* yielded 24 compounds between them, including α-muurolene, γ-muurolene, calamenene, α-bergamotene, α-copaene, α-cubebene, methylthymol, neral, geranial and guaiol (Craveiro A.A. *et al.*, 1986).

Several flavonoids from Asteraceae have been identified. Umbelliferone (7-hydroxycoumarin) from *Eupatorium angustifolium*, 5,7,8-trimethoxyflavone, 7-hydroxy-3,5,8-trimethoxyflavone and 3,5,7,8-tetramethoxyflavone from *Achyrocline satureoides* var *albicans*; of cirsimaritin from *Baccharis eleagnoides*; and of genkwanin from *Symphyopappus polystachyus* (Mesquita A.A.L. *et al.*, 1986).

The aerial parts of *Greenmaniella resinosa* Sharp. when extracted with a MeOH-EtOH-petrol mixture yielded, in addition to known compounds, 22 new ones: nine germacranolides, seven eudesmanolides, five guaianolides and a bisabolene derivative (Zdero C. *et al.*, 1987).

An investigation of South African *Senecio* species resulted in the identification of three new furanoeremophilanes, six compounds derived from cacalol and a dihydroeuparin derivative (Bohlmann F. and Zdero C., 1978c). These findings confirm that furanoeremophilanes are very widespread in this genus. Another South African genus *Eumorphia* has been found to contain several new furanosesquiterpenes and a new triterpene aldehyde (Bohlmann F. and Zdero C., 1978b).

The genus *Inula* with about 80-100 species represents a badly delineated complex, similar to some of the *Olearia* species. Chemical investigations have shown that there are two main groups, one containing sesquiterpene lactones, especially eudesmanolides, and one containing simple thymol derivatives. Investigation of four species has now led to the isolation of a considerable number of new sesquiterpene lactones (Bohlmann F. *et al.*, 1978).

Studies of *Bedfordia salicina* DC. roots showed the presence of new dimeric eremophilenolides, new kilevane derivatives, a kaurenic acid and several sesquiterpenes related to xanthatine (Bohlmann F. and Le Van N., 1978).

Thujone is the primary constituent of essential oils derived from a variety of plants, including *Artemisia absinnthium, A. vulgaris, Salvia officinalis* and *Tanacetum vulgare*. While oils derived from the individual species may vary in the modifying constituents which they contain, the pharmacological effects common to thujone-containing plants are generally considered to include the following: anthelmintic, psychedelic, a uterine stimulant and antidote to opium and other central nervous system depressant poisons (Albert-Puleo M., 1978).

The steam distilled essential oil yield of *Tanacetum vulgare* varies from 0.02 to 0.66%, of which some 50% is thujone, and exhibits a heterogeneous distribution indicating genetic variation. In addition, the essential oil composition of wild plants remains constant during their cultivation (Tetenyi P. *et al.*, 1975).

Some unusual compounds have been isolated from members of the Asteraceae family. Among these is 9-epi-β-caryophyllene from *Europys breviopapposus* M.D.Hend. (Bohlmann F. and Zdero C., 1978a).

The character of an essential oil has been found to be inherited. For instance poor quality of the oil of *Ocimum basilicum* var *glabratum* was found to be due to the presence of different chemical races in this variety. Three chemotypes were isolated and their inheritance studied: methyl chavicol, eugenol and camphor. The presence of eugenol, although recessive to methyl chavicol, is dominant to camphor and is expressed when the gene for methyl chavicol is absent. The gene for camphor is only expressed when the other two forms of gene responsible for methyl chavicol and eugenol are absent (Pushpangadan P. *et al.*, 1975).

An extensive survey of sesquiterpene components from Asteraceae members was undertaken by Bohlmann. Many new compounds were isolated including characteristically highly oxygenated sesquiterpenes, most of which were derived from a few widespread skeletons including the germacranes, eudesmanes, guaianes, cadinanes and bisabolanes. Pseudoguaianes are mainly found in some subtribes of Heliantheae, while eremophilanes are restricted to Senecioneae.

Isocomene was isolated from *Isocoma* sp. and modhephene from *Berkheya* sp. Both these compounds were found in *Silphium* sp. An acetoxy propellane was present in *Labium eggersii*. *Zexmenia* sp. yielded the rare valerenane skeleton, while a *Vernonia* sp. gave two new lactones, including a bourbonene derivative. In *Calea* sp., the

first sesquiterpene lactone substituted with a pinene residue was found. Lactones were found in both *Lasiolaena* sp. and *Graziela* sp. *Smallanthus* sp. gave a rare cis,cis-germacranolide, and an *Anthanasia* sp. yielded several new guaianolides and the first endoperoxide of a sesquiterpene lactone. Many other species were examined including : *Espeletiopsis, Doronium, Artemesia, Tanacetum, Senecio* and *Stevia* (Bohlmann F., 1980).

One important consequence of the elucidation of essential oil components is the capability of using this chemotaxonomic information for the purpose of sorting out certain taxonomic problems among the worlds' flora.

Composition of leaf oils in the genus *Parthenium* L. varies between species. However, similar monoterpene constituents are found in each, which is interpreted as being evidence of their relationship at a molecular level. It is assumed that the presence of the same monoterpene products in different species means that a common biosynthetic pathway is present. Of the species studied, one lacked bornyl acetate. This species also has one less chromosome and this lends credence to the postulation that the absence of bornyl acetate may be associated with the missing chromosome (Kumamoto J. *et al.*, 1985).

Work of this type has been presented from all plant families. For instance, the leaf oils of *Eucalyptus ovata* Labill. and *E. brookerana* A.M.Gray (Myrtaceae), fall into two distinct groups, which are discussed by Brooker M.I.H. and Lassak E.V., 1981.

Along the same lines, essential oils have been used to clarify the taxonomy of tribes of the Asteraceae. Sesquiterpene lactones reported to occur in genera of the Asteraceae were used to determine evolutionary polarity. A chemosystematic analysis of the type reported is a valuable tool in taxonomic work (Emerenciano V. de P. *et al.*, 1987).

The distinction of various chemotypes is illustrated by work carried out on the essential oils of the genus *Zieria* (Rutaceae). Three chemotypes of the species *Zieria smithii* were found to contain methyl eugenol (>50%), elemicin (>40%) and safrole (>50%) as major components (Southwell I.A. and Armstrong J.A., 1980).

The knowledge of essential oil compositions and variations is continually increasing, and it is the aim of this work to add to that store of information. In addition, the possibility of

cultivating a native species of the Asteraceae family (*Olearia phlogopappa*), with a view to commercial essential oil production, is examined.

III GENERAL MATERIALS AND METHODS

1.1 AGRONOMIC CONSIDERATIONS

1.1a MULTI-LOCATION CLONAL PERFORMANCE TRIALS

Two trial sites were selected on the basis of their proximity and availability. Both were located on sandy loam river flats, with access to irrigation water. The two sites, at Ouse and Bushy Park, had to be established independently due to insufficient planting material.

From each of six sites around Tasmania one plant was chosen as the stock plant for cloning. The clones were used in the trials at Ouse and Bushy Park. The six source sites were Elephant Pass (EP), Buckland (BU), Paradise Plains (PP), Mount Wellington (MW), Eaglehawk Neck (EN) and Great Lake (GL). Plants originating from each of these areas are shown in Plate 1, whilst an example of Olearia in flower is shown in Plate 2.

Planting dates were Bushy Park : 1st July 1986 and Ouse : 12th November 1986. Both trials were set up as follows:

Ground preparation included spraying with Roundup (@ 4 l/ha with wetting agent) and Simazene (@ 1.8 l/ha) and cultivation some two months prior to planting out. Irrigation was supplied as required.

Additional weed control during the season was maintained by hand-hoeing.

The layout consisted of a randomised block design. The six clones were labelled A to F thus:

A Great Lake (GL 42)

B Paradise Plains (PP 6)

C Elephant Pass (EP 26)

D Mount Wellington (MW)

E Buckland (BU 01)

F Eaglehawk Neck (EN)

Four blocks were planted out. Each block comprised six plots of ten plants. Each clone was represented in each block, thus:

PLATE 1

Great Lake GL

Buckland BU

Eaglehawk Neck EN

Paradise PLains PP

Elephant Pass EP

Mount Wellington MW

Olearia phlogopappa in flower

PLATE 2

BLOCK							
	I	C	B	D	E	A	F
	II	E	C	F	A	B	D
	III	F	E	D	B	C	A
	IV	B	A	C	E	F	B

The plants were arranged in a 0.5 x 0.5 m triangular spacing configuration. Each plot was separated from the next by two guard plants, with additional guard plants around the perimeter of the entire trial plot. The two sites are depicted in Plates 3 and 4.

1.1b MEASUREMENTS

All plants were assessed for height, diameter of stem at 3 cm above ground, and width, at monthly intervals. The height of the plants was measured using a metre rule, from the ground surface to the highest point of the foliage. The width was taken across the plant perpendicular to the direction of the row.

Each plant stem was marked with white paint at the start of the trial. Since the plants were only some eight or nine centimeters high when they were planted out, the marks were placed low on the stem (3 cm or so from ground level). It was at these points that stem diameter was monitored.

In addition samples were collected for steam distillation and/or solvent extraction. These samples were collected from the current growth, simulating material that would be collected during a harvest. The methods for the oil extraction and analysis are given below.

OUSE

PLATE 3

BUSHY PARK

PLATE 4

1.2 ESSENTIAL OIL PROCESSING

1.2a STEAM DISTILLATION

Samples of leaf and stem material (500-900 g) were collected and placed in a specially constructed stainless steel basket, and lowered into the round-bottomed distillation vessel. The distillation apparatus is shown in Plate 5. The basket holds the plant material above a volume of water, from which steam is generated. Steam rises up through the herb, carrying the oil with it.

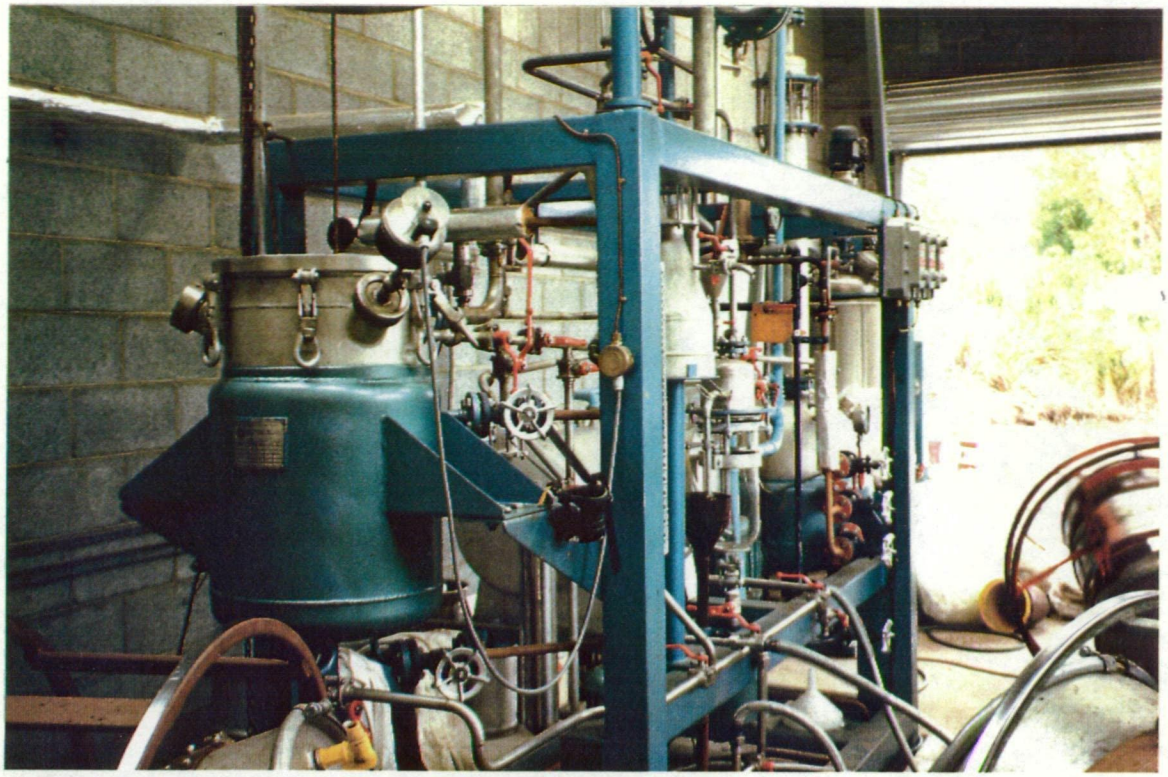
The overall process is one of cohobation, where the oil/water mixture passes through the condenser and is collected in a U-tube receiver. The collector has a side arm that allows the return of distillation water to the vessel. At the end of the run the oil was taken up using a pipette and placed in a vial. After weighing, the oil was dried with anhydrous sodium sulphate.

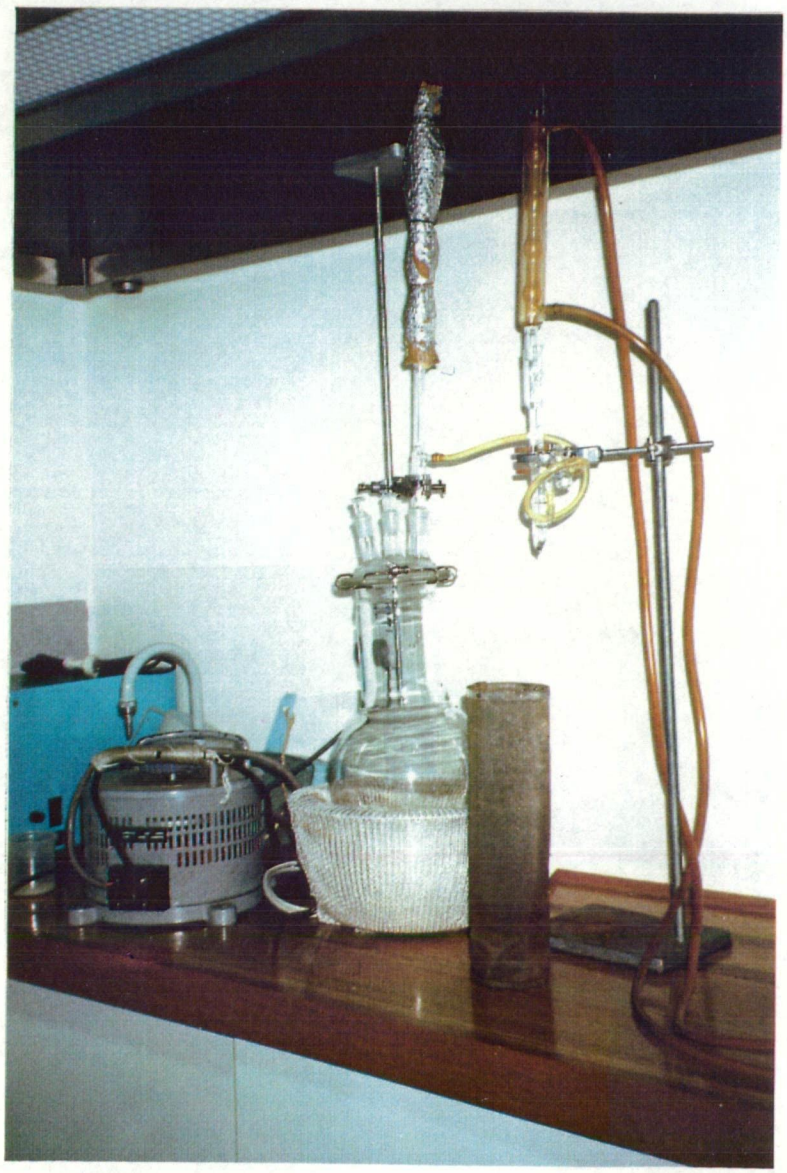
Since the harvesting of Olearia from the trial sites resulted in considerable volumes of plant material which required processing, a larger scale distillation apparatus was necessary. To this end, a semi-commercial still was used. This unit is located at the Horticultural Research Centre, and is shown in Plate 5.

1.2b SOLVENT EXTRACTION

For small scale extractions some 2 to 3 g of leaf and stem material was collected. Each sample was initially frozen with liquid nitrogen. The frozen material was transferred to a porcelain mortar for grinding. An aliquot of redistilled hexane was added, along with 1.0 ml of standard solution (0.5 g C_{18} in 200 ml). The addition of approximately 0.5 g anhydrous sodium sulphate assisted the grinding process.

The hexane/oil mixture was taken up with a pipette and transferred to a glass vial. Approximately 0.02 g charcoal was added to decolourise the solution. This level of charcoal has been found sufficient to decolourise the solution without the selective absorption of components such as caryophyllene, terpineol and terpinene-4-ol (Burgess T.I., 1986). The resultant clear solution was transferred to a vial fitted with a cap which has a rubber





Above: Semi-commercial scale distillation unit.

Left: Distillation apparatus as used in the laboratory.

PLATE 5

insert, suitable for automated gas chromatography (gc) analysis.

1.3 GLASSHOUSE TRIAL - MOISTURE STRESS

The moisture stress experiment was conducted in a glasshouse at the Horticultural Research Centre. The temperature range was from 12°C at night to 25°C during the day.

Cuttings of the MW type were established in solution culture soon after the appearance of roots. The plants were left to adjust to the conditions and resume growth. After 10 days, and the emergence of new white roots, the plants could be transferred to the experimental pots. The containers used were black polythene buckets (5 l capacity), which were resistant to degradation in ultra violet light.

The experiment was set out in a randomised block design. There were four blocks with each block containing each of the five treatments; three plants per treatment. The polyetheylene glycol,(PEG), (molecular weight 1000), was added to the treatment solutions in such a way as to achieve five levels of osmotic stress. The concentration of PEG was determined from the method used by Cheesman *et al.* (1965), who determined the relationship between the amount of PEG 1000 and the osmotic pressure in atmospheres. The graph of the relationship is shown in Figure III.1.

The amounts of PEG required and the osmotic pressure generated, are shown below:

MOISTURE TENSION (atm)	WT. OF PEG (g)
0.4	0
1.0	75
2.0	195
3.0	323
4.0	450

Normal Hoaglands solution was used as the basic growing medium and also for the control plants (0 g PEG). The Hoaglands solution comprised the following:

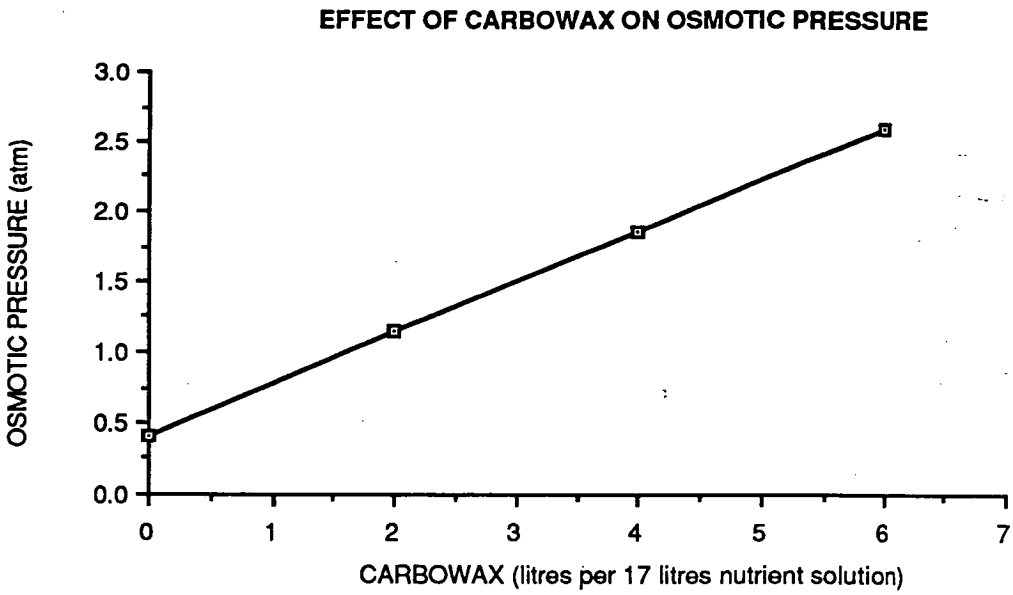

EFFECT OF CARBOWAX ON OSMOTIC PRESSURE
OSMOTIC PRESSURE (atm)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
7
CARBOWAX (litres per 17 litres nutrient solution)

FIGURE III.1

NORMAL HOAGLANDS

a)	Macronutrients	ml/l
	1M KH_2PO_4	1
	1M KNO_3	5
	1M $Ca(NO_3)_2$	5
	1M $MgSO_4$	2
b)	Micronutrients	g/l
	H_2BO_3	2.86
	$MnCl_2.4H_2O$	1.81
	$ZnSO_4.7H_2O$	0.22
	$CuSO_4.5H_2O$	0.08
	$H_2MoO_2.H_2O$	0.02

(Walker R.R. (1971))

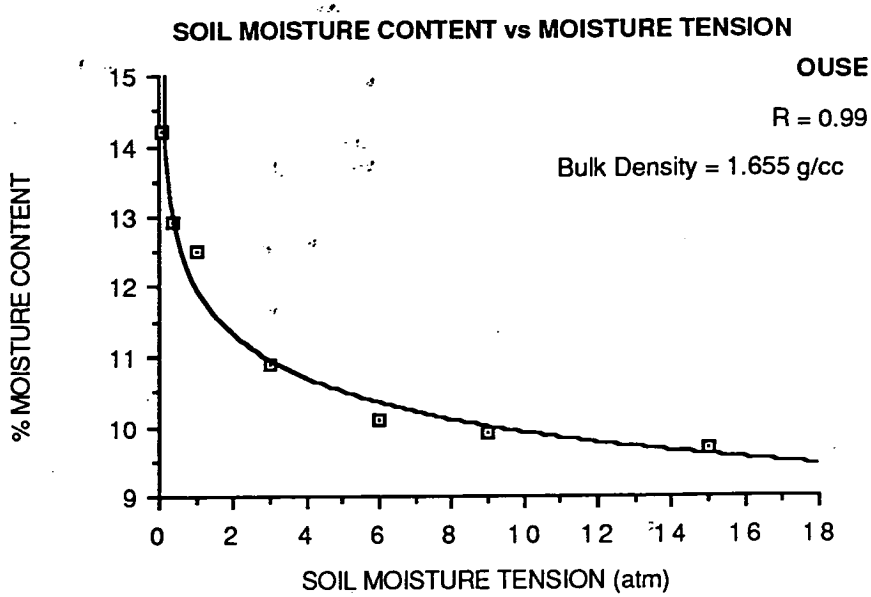
The micronutrients were made up to 1 l then 1 ml of the solution was added to each litre of normal Hoaglands. The solution was complete with the addition of 1 ml FeEDTA (3.28 g/l) per litre.

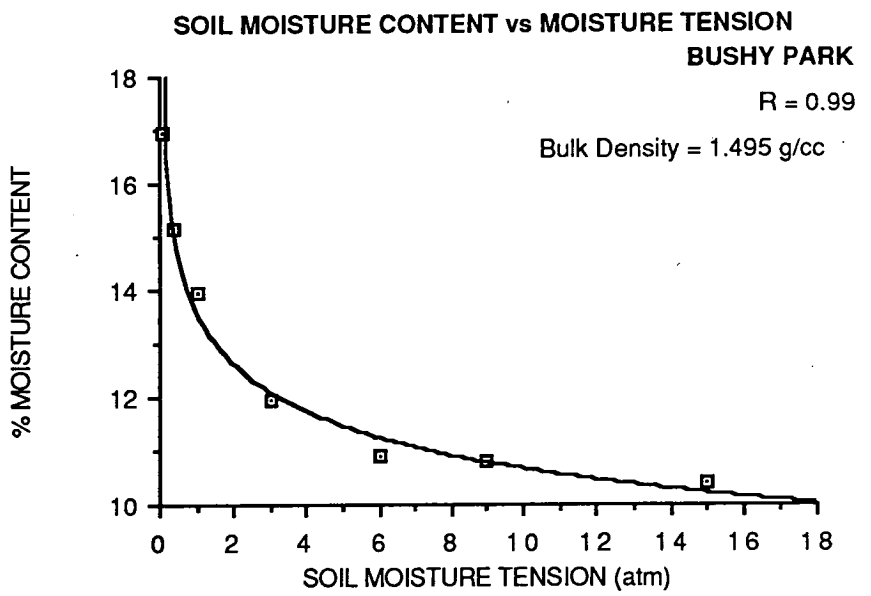
In order to be able to relate the results of the solution culture experiment to the situation in the field, a series of trials were run to determine soil moisture versus osmotic tension in Bushy Park, Ouse and Fern Tree soils.

A pressure chamber (Soil Moisture Equipment Co., Santa Barbara Calif. 15 Bar Ceramic Plate Extractor Cat.No. 1500) was used to determine the soil moisture at 0.1, 0.4, 1.0, 3.0, 6.0, 9.0 and 15.0 atmospheres. The graphs showing the relationship are given for all three soil types in Figure III.2.

The status of the plants in solution culture, as given by the stomatal resistance, was monitored using a Li-Cor LI-65 Autoporometer (Lambda Instruments Corporation, Lincoln Nebraska).

The instrument was calibrated for use with Olearia leaves. The calibration plot is shown in Figure III.3. The stomatal resistance of plant leaves changes throughout the day (Clarke J.M. and McCraig T.N., 1982). Therefore, a series of measurements of stomatal resistance was made from 5:50 am to 1:25 pm, to determine the optimum time to carry out diffusive resistance measurements during the moisture stress trial. Three plants were used: a well established plant in solution culture, a newly established one, also in solution culture, and a plant growing in a sand/peat medium. The results are shown in Figure III.4. The lower the diffusive


SOIL MOISTURE CONTENT vs MOISTURE TENSION
OUSE
R = 0.99
Bulk Density = 1.655 g/cc
% MOISTURE CONTENT
15
14
13
12
11
10
9
0 2 4 6 8 10 12 14 16 18
SOIL MOISTURE TENSION (atm)


SOIL MOISTURE CONTENT vs MOISTURE TENSION
BUSHY PARK
R = 0.99
Bulk Density = 1.495 g/cc
% MOISTURE CONTENT
18
16
14
12
10
0 2 4 6 8 10 12 14 16 18
SOIL MOISTURE TENSION (atm)

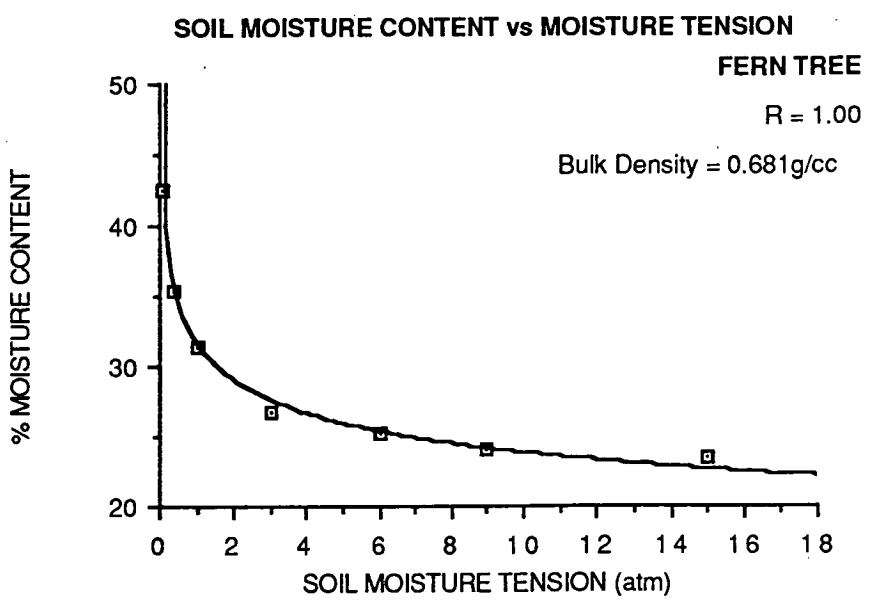

SOIL MOISTURE CONTENT vs MOISTURE TENSION
FERN TREE
R = 1.00
Bulk Density = 0.681g/cc
% MOISTURE CONTENT
50
40
30
20
0 2 4 6 8 10 12 14 16 18
SOIL MOISTURE TENSION (atm)

FIGURE III.2

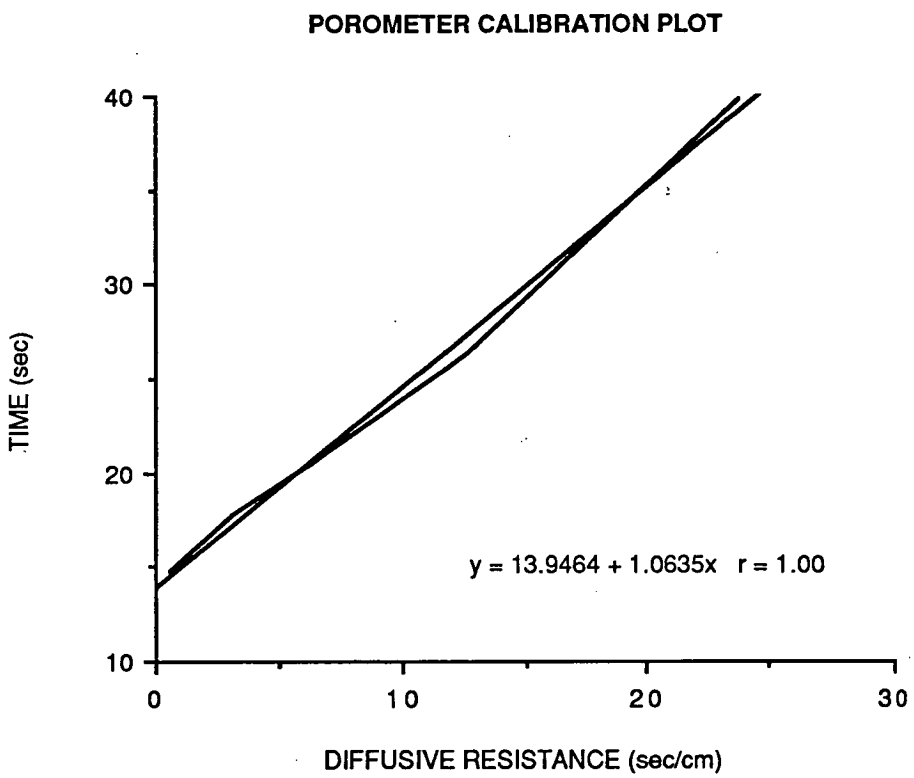

POROMETER CALIBRATION PLOT
40
30
20
10
TIME (sec)
y = 13.9464 + 1.0635x r = 1.00
0
10
20
30
DIFFUSIVE RESISTANCE (sec/cm)

FIGURE III.3

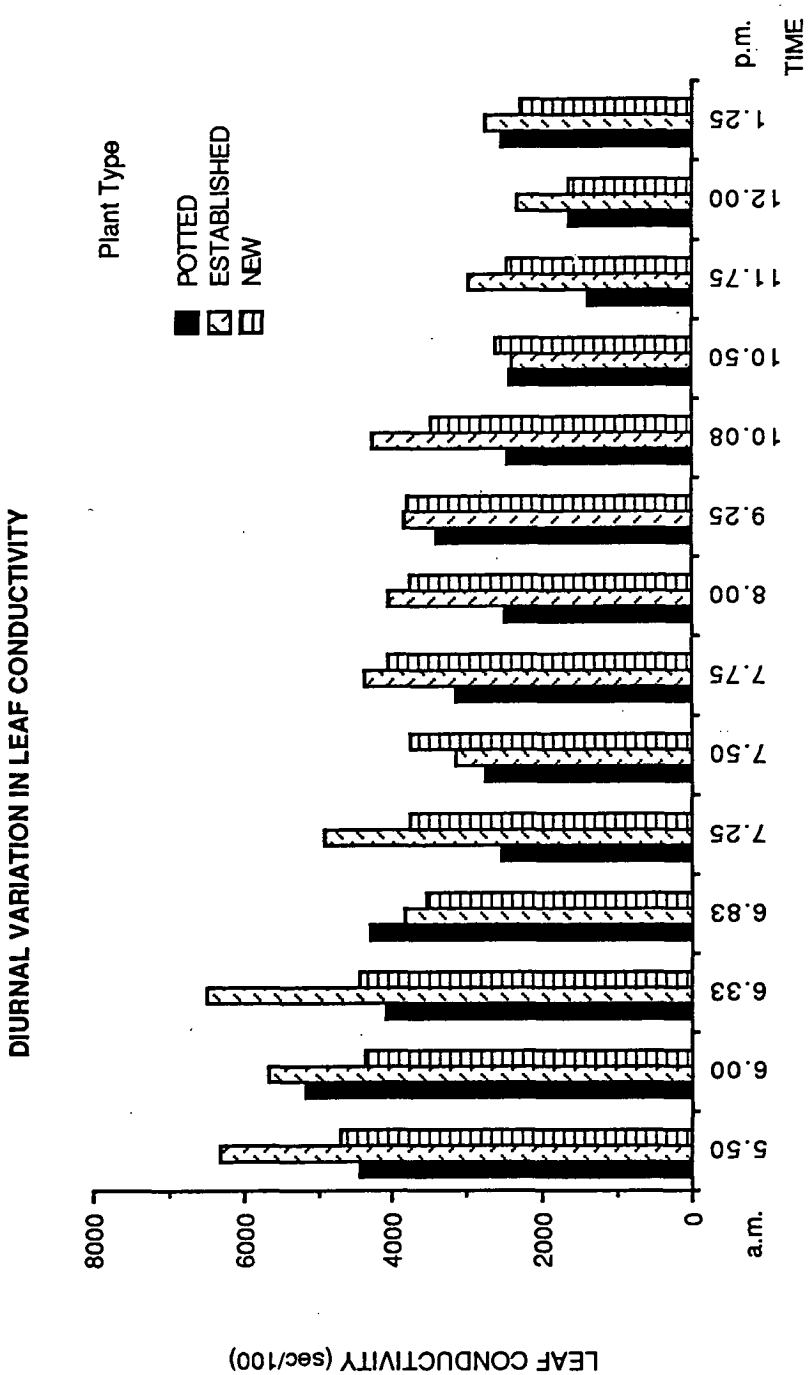

DIURNAL VARIATION IN LEAF CONDUCTIVITY
Plant Type
POTTED
ESTABLISHED
NEW
LEAF CONDUCTIVITY (sec/100)
8000
6000
4000
2000
0
a.m.
5.50
6.00
6.33
6.83
7.25
7.50
7.75
8.00
9.25
10.08
10.50
11.75
12.00
1.25
p.m.
TIME

FIGURE III.4

resistance, that is, the more open the stomata, the higher the rate of photosynthesis. The maximum rate of photosynthetic activity is the optimum time for stomatal resistance measurements. In this case, therfore, measurements were taken between 9.00 a.m. and 12 noon.

Since the leaf water potential and the stomatal resistance are directly related (Allen S.G. and Nakayama F.S., 1988), the porometer readings were taken as a guide to the severity of the imposed stress.

1.4 SCANNING ELECTRON MICROSCOPY

The leaf specimens were mounted on electron microscope stubs using double sided tape. They were arranged so that all three replicates of one plant type were accessible for observation with the scanning electron microscope (sem) at the same time. There was not need for fixation of the samples, due to the short time delay between mounting and observation. This 'fresh' technique also discouraged the formation of artifacts.

A Blazers Sputter Coater was used to coat the samples with approximately 20nm of gold. A Phillips 505 Scanning Electron Microscope, set at 15 kV, was used to observe the specimens, and electron micrographs were recorded with a Rolex 120 land camera.

1.5 GAS CHROMATOGRAPHY

1.5a PREPARATIVE

Preparative gas chromatography was performed using the following system:

Glass column: 2m x 5mm (i.d.)
Packing: 10% SE 30 on Chrom W (AW DCMS)
Mesh: 80/100
Temperature program : 80 - 250°C at 5°C per minute
Detector temperature : 250°C
Air pressure : 76 kPa
Hydrogen pressure : 117 kPa
Carrier gas (Nitrogen) pressure: 160 kPa
Flow rate : 25 ml/min
Injection volume: 10µl neat oil
Splitter: 25 : 1

1.5b ANALYTICAL

A Hewlett Packard 5880A Gas Chromatograph was used to analyse all routine samples. The following operating conditions were employed:

Column: BP1 12 m x 0.5 mm
Program: 50(1) to 230(5) at 6° C/min
Carrier gas: Helium
Carrier gas pressure: 7 psig
Detector: FID
Detector temperature: 240° C
Injector temperature: 240° C
Injection volume: 1µl
Split injection: 20:1
Sample solution: 5µl oil per ml hexane

In many instances, the yield of essential oil was determined using a C_{18} internal standard. A known amount was added to the extract and the percentage yield was determined as follows:

$$\% \text{ yield} = \frac{\frac{100 - \% \text{ I.S.}}{\% \text{ I.S.}}}{\text{fresh weight}} \times 100$$

It should be mentioned here that compositional data is reported in terms of percentage total peak area, and as such one may assume that the detector response is equivalent from one component to the next (Clark R.J. and Menary R.C., 1984).

1.6 GAS CHROMATOGRAPHY/FOURIER TRANSFORM INFRA-RED ANALYSIS

Infra-red spectra were accumulated using a Digilab FTS-20E instrument, with a GC/IR light pipe attachment. This was coupled to the HP 5880A GC.

Column: Chrompack CP-SIL-5CB 25 m x 5 mm
Program: 50(1) to 280(15) at 10°C/min
Head pressure: 5 psig
Detector temperature: 290°C
Injector temperature: 290°C
Light pipe temperature: 290°C
Splitless injection
Injection volume: 0.1µl neat oil, or equivalent concentration in $CHCl_3$

1.7 GAS CHROMATOGRAPHY/MASS SPECTROMETRY

Essential oil samples were analysed using a Hewlett Packard 5890 Gas Chromatograph coupled to a Hewlett Packard 5970 series Mass Spectrometer using the following conditions:

Detector: MSD
Column: BP1 12 m x 0.5 mm
Program: 50°C to 230°C at 6°C/min
Head pressure: 7 psig

1.8 NUCLEAR MAGNETIC RESONANCE SPECROSCOPY

The 300 MHz ^{1}H and ^{13}C nuclear magnetic resonance (nmr) spectra were obtained on a Bruker AM300 spectrometer. Tetramethylsilane was used as the internal standard, at 0 ppm, during the collection of all ^{1}H and ^{13}C NMR spectra. All 2D nmr experiments were performed using standard Bruker microprograms. The ^{1}H and ^{13}C 90° pulses were 13.3 and 4.1 microseconds, respectively for the 5 mm probe.

The DEPT.AUR microprogram was used routinely for editing of the ^{13}C nmr spectra, in order to determine the degree of protonation of the observed resonances. The unambiguous assignment of ^{1}H and ^{13}C nmr spectra was made possible by the use of two basic 2D nmr experiments, namely the COSY.AUR and the XHCORR.AUR Bruker pulse programs.

The COSY.AUR pulse sequence was used with a 45° mixing pulse which resulted in suppression of diagonal peaks. This experiment enabled the proton-proton couplings networks to be efficiently determined.

Because of the crowded nature of some of the ^{1}H nmr spectra, an alternative experiment (COSYDEC.AUR) was used. This yielded 2D spectra ^{1}H decoupled in the F1 dimension and was found to be particularly useful. The coupling information was still present in the F2 dimension, enabling coupling constants to be measured.

The ^{1}H - ^{13}C correlations (XHCORR), were determined using two XHCORR.AUR pulse programs. The two variable delay periods in the pulse sequence were tuned to detect ^{1}J ^{1}H-^{13}C couplings.

The general principles on which these nmr experiments were based, and the rationale behind their use for structural elucidation, are well documented in the monograph by Croasmun R. and Carlson R.M.K, 1987. Deuterochloroforme was the sample solvent.

1.9 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Reversed phase high performance liquid chromatography was used to separate essential oil components. Strack D. *et al.* described a method employing acetonitrile/water as the mobile phase on a C_8 or C_{18} reversed phase column. The excellent resolution of a very complex terpenoid mixture using a linear gradient from 60% to 100%

acetonitrile in less than one hour, prompted the use of a similar method. (Strack D. *et al.*, 1980).

1.9a GENERAL

The High Performance Liquid Chromatography (HPLC) work was carried out using the following equipment:

Waters 6000A pump (x2)
Waters 490E Programmable Multiwavelength Detector
Waters U6K Injector
Waters RCM

1.9b ANALYTICAL SCALE CHROMATOGRAMS

Due to the sparing solubility of *O. phlogopappa* oils in methanol, acetonitrile, and insolubility in water, oil samples were dissolved in tetrahydrofuran (THF, HPLC grade). The following samples were prepared for injection onto the column:

MW, GL, PP, EP, EN, BU

Some adjustment of the acetonitrile/water gradient, with respect to flow rate and gradient, was necessary to achieve suitable conditions for optimal separation of components. The following conditions were settled upon:

Column: RadpakμBondapak C_{18} 8 mm x 10 cm (Part No. 85721)
Solvent A: HPLC grade acetonitrile
Solvent B: filtered distilled water
Gradient: 36% to 100% (Solvent A) in 40 minutes
Flow rate: 2 ml/min
Detection: 220nm, 3.0 AUFS
Chart speed: 6 min/cm
Sample solution: 100 mg/ml
Injection volume: 3μl

The preparation of a saturated hexane/acetonitrile solvent was carried out as follows:

500 ml hexane and 50 ml acetonitrile were shaken together in a separating funnel. The lower layer was discarded and the hexane was then diluted as appropriate, with hexane, for use with the hplc.

1.9c PREPARATIVE SCALE SEPARATION

Column:μBondapak C_{18} 7.8 mm x 30 cm (Part No. 84176) or
: AMICON LC 22 mm x 250 mm (Catalog No. 95193) or
: Brownlee Labs RP-18 Aquapore cartridge 10 mm x 220 mm
Solvent A: HPLC grade acetonitrile
Solvent B: filtered distilled water
Gradient: 36% to 100% (Solvent A) in 40 minutes
Flow rate: 6 ml/min
Detection: 230 nm, 3.0 AUFS
Chart speed: 6 min/cm
Sample solution: 100 mg/ml
Injection volume: 100μl

Other solvent systems were also used in the final stages of fraction purification, namely:

SOLVENT A	SOLVENT B
Methanol	Water
Tetrahyrdrofuran	Water
iso-propanol	Water

IV. RESULTS AND DISCUSSION

1. WILD POPULATIONS

1.1a PRELIMINARY CONSIDERATIONS

Initially, a broad scan of populations of *Olearia phlogopappa* was undertaken with the aim of locating suitable clonal material for commercially orientated growth trials.

A subjective survey of several populations of *Olearia phlogopappa* around eastern Tasmania revealed that not all forms of this plant are odoriferous. Populations without distinctive aroma were found at Snug Plains, Collinsvale, Mt. Dromedary, Glendevie, Lune River, Lauderdale and Murdunna. Conversely, plants from Great Lake, Paradise Plains Elephant Pass, Mount Wellington, Buckland and Eaglehawk Neck were distinguished by the strongly odorous essential oil released when the leaves were crushed between the fingers.

Further, among plants of any one population there seemed to be organoleptic variation in the essential oil, as well as differences in their morphology. Consequently, three sites were selected where there were sufficient numbers of plants to sustain a small-scale morphological survey. This, coupled with essential oil yield data, was aimed at determining whether any of the selected morphological characters could be used to predict potential oil yield in the field.

Paradise Plains, Elephant Pass and Great Lake were surveyed over a period of nine months. At each of these three sites the population was large enough to enable fifty plants to be selected for sampling at random. Each was more than three metres distant from any other chosen plant. Morphological characters such as height, lamina width and length were measured. Some characters (namely lamina width, lamina length and the number of serrations per half leaf margin), were replicated within plants. Also, samples were taken for steam distillation and gas chromatographic analysis, percentage dry matter evaluation and examination by scanning electron microscope to determine the number of oil glands per square millimeter. An analysis of variance (ANOVA), was performed separatedly on the data concerning oil glands. Thus, any significant variation in gland size existing between plants from the same site could be identified. The gland number data was collated

and used in the ANOVA determinations of morphological characters.

The locations of these three areas, (as well as that of the sources of the other three clones from which source material originated), are shown on the map in Figure IV.1.1, and the altitudes and grid references are presented in Table IV.1.1.

TABLE IV.1.1

ALTITUDE AND GRID REFERENCES OF OLEARIA SOURCE SITES

SITE	ALTITUDE (m)	GRID REFERENCE
A Great Lake	1100	146 41'30'':42 00'15''
B Paradise Plains	840	147 42'30'':41 20'10''
C Elephant Pass	420	148 13'00'':41 38'15''
D Mount Wellington	650	147 14'25'':42 56'00''
E Buckland	320	147 39'50'':42 36'30''
F Eaglehawk Neck	0	147 55'20'':43 01'00''

1.1b OIL GLANDS

The adaxial leaf surfaces of plants from three sites were examined to determine the variation in the number and size of oil secreting glands.

Leaf samples were prepared for the scanning electron microscope as outlined in Materials and Methods, and the diameter of the oil glands carried on the adaxial leaf surface was examined. Four clones from each of the Great Lake, Elephant Pass and Paradise Plains sites were selected at random for this investigation. A list of clones, their mean oil gland diameter and standard errors are listed in Table IV.1.2. The ANOVA and comparisons between clones are presented as Appendix A. These results are shown graphically in Figure IV.1.2.

Each of the three groups are significantly different from each other in respect of gland size. Taking each group separately, the Great Lake samples contained three plants which had glands of similar size and one (GL 32) which had significantly (at $p \leq 0.05$) larger glands (Appendix A ANOVA). Amongst the Elephant Pass plants, EP 12, EP 27 and EP 26 all had significantly ($p \leq 0.05$) smaller glands than EP 44. The Paradise Plains plants also fell into two groups with PP 6 and PP 10 having significantly larger ($p \leq 0.05$) glands than PP 22 and PP 34. (Appendix A)

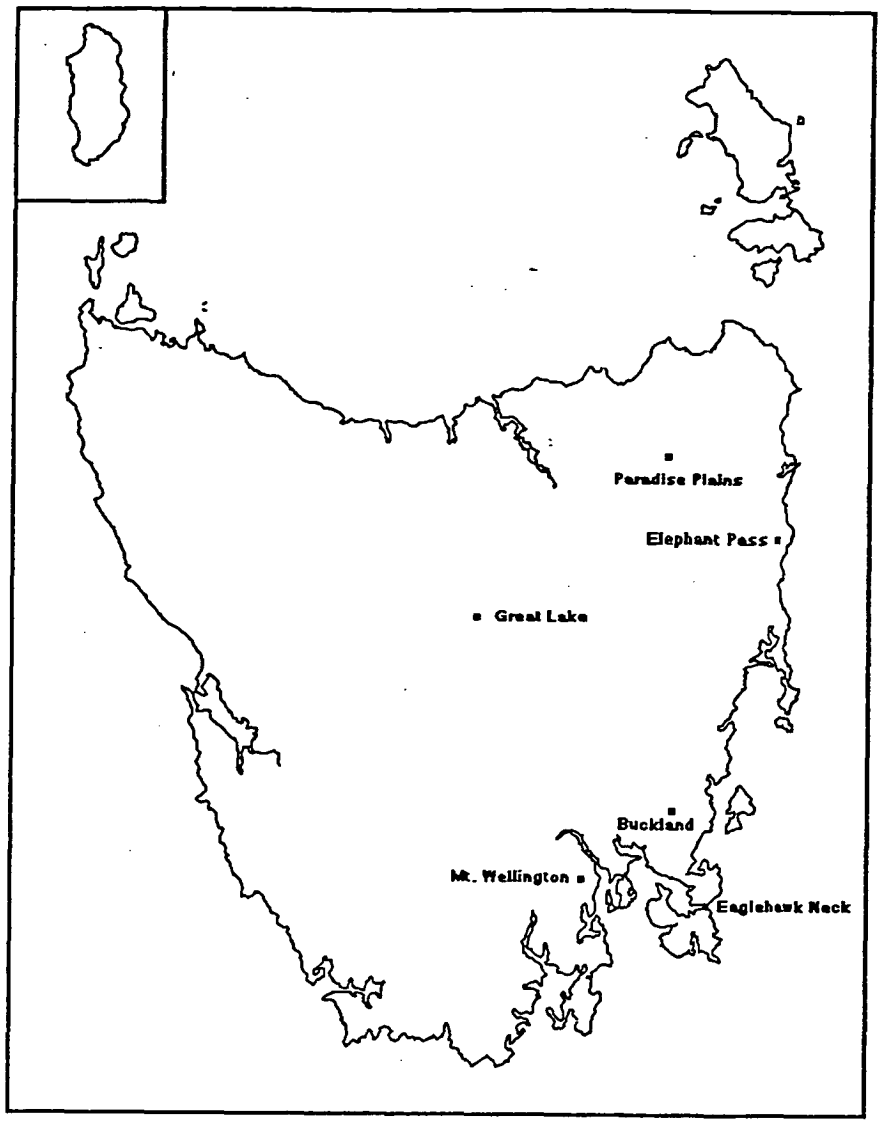


FIGURE IV.1.1

MAP OF TASMANIA

SHOWING LOCATIONS FROM WHICH CLONES ORIGINATED

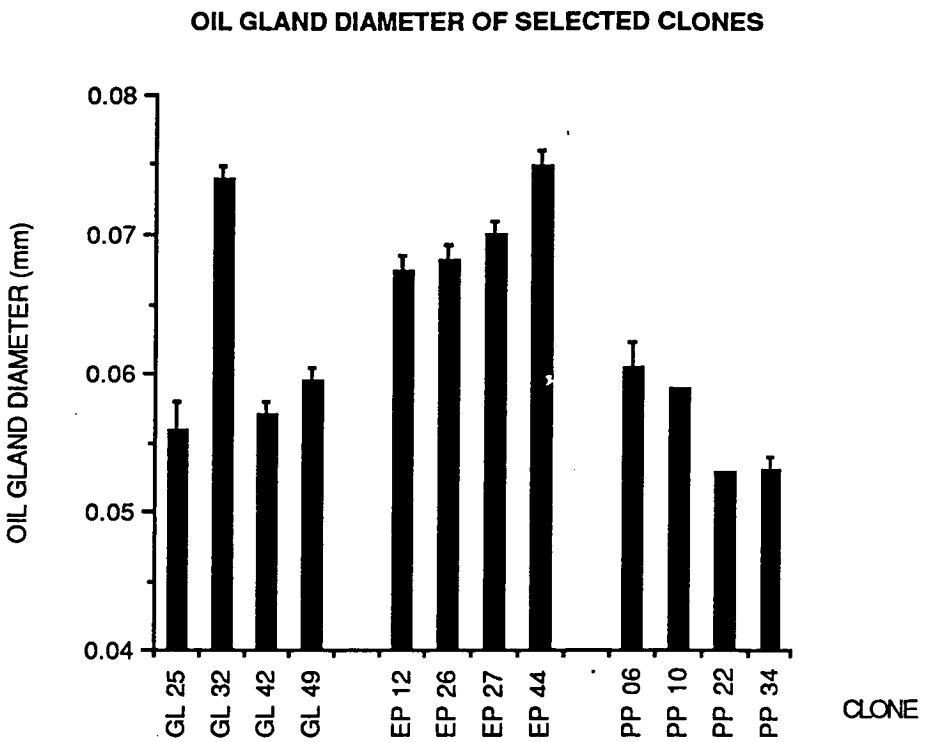

OIL GLAND DIAMETER OF SELECTED CLONES
OIL GLAND DIAMETER (mm)
0.08
0.07
0.06
0.05
0.04
GL 25
GL 32
GL 42
GL 49
EP 12
EP 26
EP 27
EP 44
PP 06
PP 10
PP 22
PP 34
CLONE

FIGURE IV.1.2

A further study was carried out, using the same semi preparative methods, in which four leaves were taken at random from eleven plants from Elephant Pass, twelve from Paradise Plains and seven from the Great Lake site. Each leaf was treated as a replicate when examined. The number of oil glands per unit area were counted, and the result converted to number per square millimeter. The data is presented as Appendix B, and used in the determination of characters correlated with percentage essential oil yield.

TABLE IV.1.2

MEAN GLAND DIAMETER FOR CLONES FROM THREE SITES

($n = 9$, $p \leq 0.05$)

CLONE	MEAN DIAMETER (mm)	S.E.
GL 25	0.0559	0.001
GL 32	0.0739	0.001
GL 42	0.0569	0.001
GL 49	0.0594	0.002
Mean	0.0615	
PP 6	0.0603	0.002
PP 10	0.0588	0.000
PP 22	0.0529	0.000
PP 34	0.0530	0.001
Mean	0.0562	
EP 12	0.0674	0.001
EP 26	0.0682	0.001
EP 27	0.0700	0.001
EP 44	0.0750	0.001
Mean	0.0715	

1.1c VARIATION IN NATURAL STANDS OF OLEARIA SPECIES

There are three main areas where an indication of the variation present in the population can be found. The first lies in between-site differences. Separate ANOVAS are performed on characters with no replication and those with replication, based on plant means.

A summary of the results is shown in Table IV.1.3.

TABLE IV.1.3

BETWEEN-SITE VARIATION IN POPULATIONS OF OLEARIA SP.

Non-Replicated Characters:	d.f.	F value	p	
Height	(2,30)	3.956	$0.025<p\leq0.05$	*
Mean lamina width	(2,28)	47.145	$p\leq0.0001$	***
Mean lamina length	(2,28)	97.683	$p\leq0.0001$	***
No. stems/plant	(2,28)	5.925	$0.005<p\leq0.01$	**
% Dry Matter	(2,28)	19.455	$p\leq0.0001$	***
% Oil Yield D.M.B.	(2,27)	0.823	$p>0.25$	ns
No. glands/mm	(2,26)	5.133	$0.01<p\leq0.025$	*
Mean serrations/leaf	(2,28)	19.13	$p\leq0.0001$	***
Replicated Characters:				
Lamina width	(2,90)	49.778	$p\leq0.0001$	***
Lamina length	(2,90)	161.073	$p\leq0.0001$	***
Length/Width	(2,90)	5.191	$0.005<p\leq0.01$	**
Serrations/leaf	(2,90)	60.527	$p\leq0.0001$	***

The results show that there is a highly significant difference between sites for most morphological characters, especially any leaf size characteristics, but there is no significant difference in percentage essential oil content on a dry matter basis. To some extent the number of glands/leaf is also less variable.

A further aspect of variation arises between plants within sites. This was tested by analysing the variation of unreplicated characters for each site. These characters were lamina length, lamina width, the ratio of length to width and the number of serrations per half leaf margin. All of the characters tested showed significant variation between plants within sites.

Table IV.1.4 shows the between plant, within site variation between plants. Thus, at each site even at this level, there is a significant variation between plants.

TABLE IV.1.4
BETWEEN PLANT VARIATION
d.f. : (between plants, within plants)

SITE	d.f.	F value	p	
Elephant Pass	(3,140)	240.228	$p \leq 0.0001$	***
Paradise Plains	(3,128)	192.574	$p \leq 0.0001$	***
Great Lake	(3, 92)	247.366	$p \leq 0.0001$	***

1.1d CORRELATIONS BETWEEN MORPHOLOGICAL CHARACTERS AND ESSENTIAL OIL YIELD

If one ignores the site differences and looks at pooled values to determine correlations between characters, one finds the following. There are no significant correlations amongst oil yield, glands/area and percentage dry matter, see Figures IV.1.3 and IV.1.4. However, some significant ($p \leq 0.05$) correlation exists between oil yield or number of glands and the proportion of some major monoterpene and sesquiterpene components of the oil. These were designated as A to E:

A α-pinene
B linalool
C germacrene-D
D bicyclogermacrene
E β-eudesmol

The correlations are shown in Table IV.1.5. There were only two components which showed any correlation with either percentage oil yield or the number of oil glands per square millimeter of leaf surface. The ratio of component B to E is significantly, positively correlated with percentage oil yield, whilst component E is negatively correlated with this character. component B alone has a significant positive correlation with the number of oil glands per mm^2.

From this survey there appears to be no simple method whereby a plants' morphology can be examined and its potential oil yield estimated. Table IV. 1.6.

Thus, a variation exists in the species, but whether it is

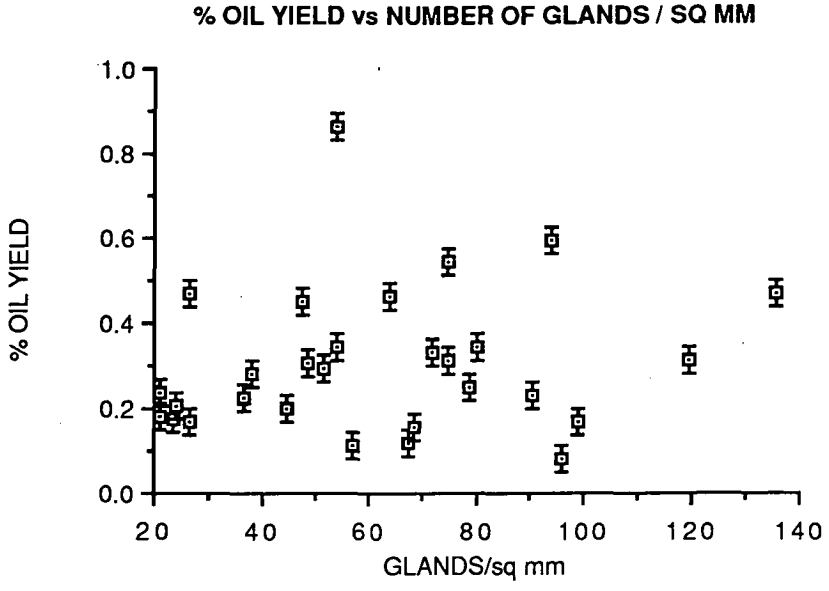


FIGURE IV.1.3(a)

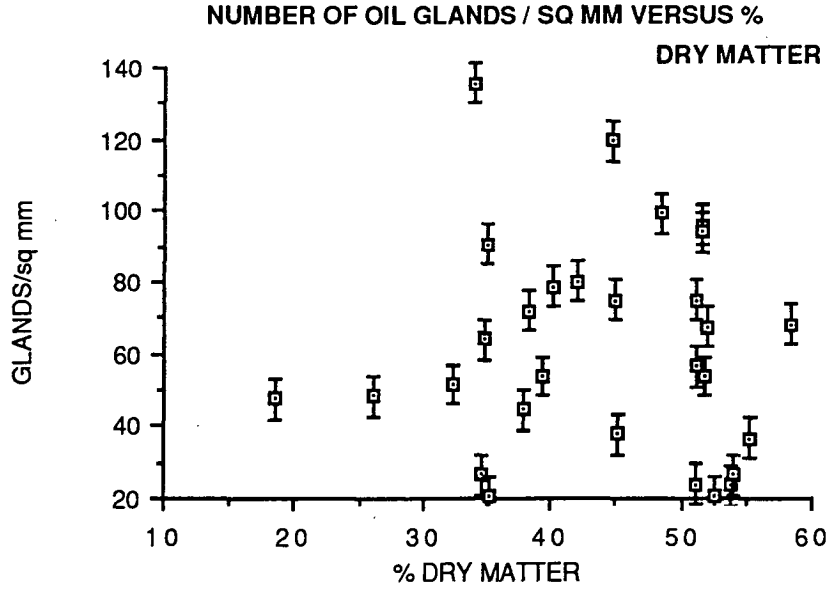
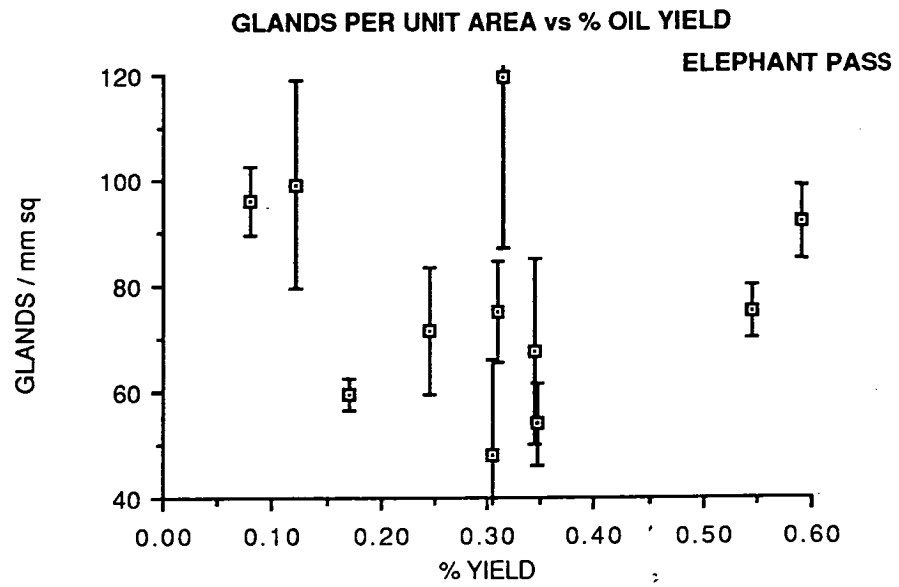
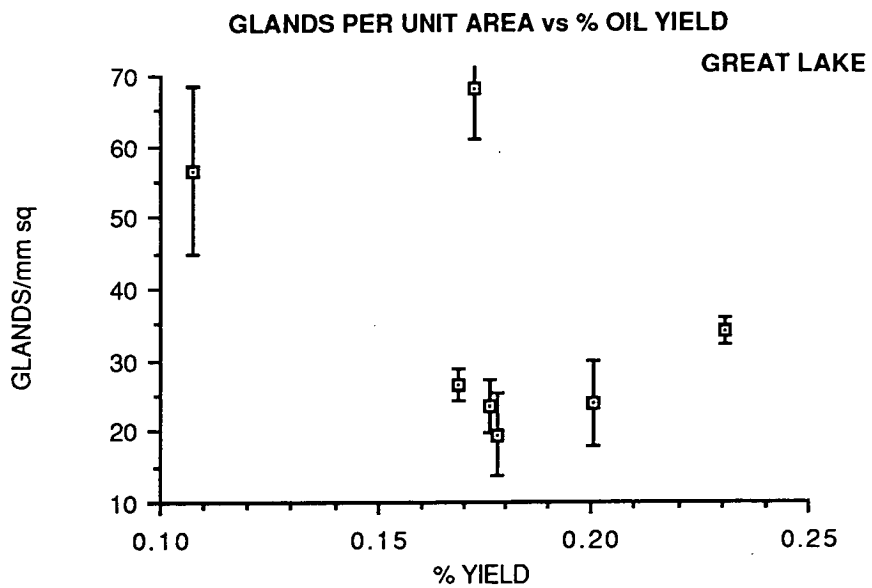


FIGURE IV.1.3 (b)


GLANDS PER UNIT AREA vs % OIL YIELD
ELEPHANT PASS
GLANDS / mm sq
120
100
80
60
40
0.00
0.10
0.20
0.30
0.40
0.50
0.60
% YIELD


GLANDS PER UNIT AREA vs % OIL YIELD
GREAT LAKE
GLANDS/mm sq
70
60
50
40
30
20
10
0.10
0.15
0.20
0.25
% YIELD

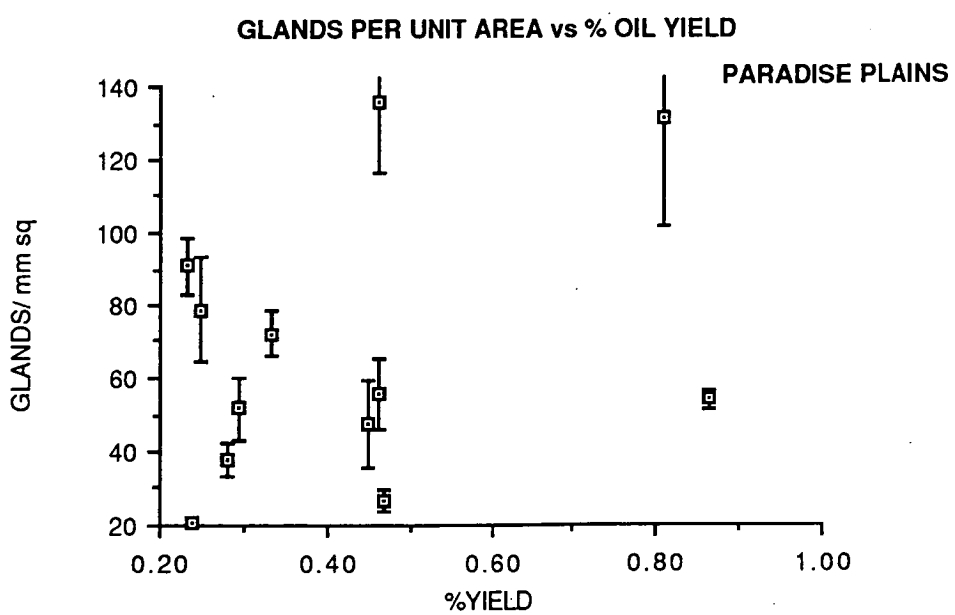

GLANDS PER UNIT AREA vs % OIL YIELD
PARADISE PLAINS
GLANDS/ mm sq
140
120
100
80
60
40
20
0.20
0.40
0.60
0.80
1.00
%YIELD

FIGURE IV.1.4

genetic or environmental was not determined at this stage. A multi-location trial was established at Ouse and Bushy Park to clarify the source of variation, and to determine the effects of growing site on clonal performance. In addition, the effect of harvesting was investigated.

TABLE IV.1.5

CORRELATION COEFFICIENTS FOR OIL YIELD AND GLANDS/mm^2

WITH COMPONENTS A TO E, IRRESPECTIVE OF SITE

X_1 : % Oil Yield X_2 : Number of glands/mm^2

COMPONENT	Corr.(r)	r^2	d.f.
A	-0.089	0.008	26
	0.025	0.042	25
B	0.127	0.016	26
	0.341 *	0.117	25
C	-0.179	0.032	28
	0.245	0.060	27
D	-0.211	0.045	28
	0.176	0.031	27
E	-0.559 *	0.313	10
	-0.150	0.022	10
RATIO A/B	-0.133	0.180	26
	-0.041	0.022	25
RATIO A/C	-0.085	0.007	28
	-0.266	0.071	27
RATIO A/D	0.040	0.002	28
	0.011	0.0001	27
RATIO A/E	0.387	0.150	10
	-0.090	0.008	10
RATIO B/C	-0.063	0.004	28
	-0.029	0.001	27
RATIO B/D	0.183	0.033	28
	0.370	0.137	27
RATIO B/E	0.643 *	0.413	10
	0.382	0.146	10
RATIO C/D	-0.103	0.011	28
	0.259	0.067	27
RATIO C/E	0.027	0.073	10
	-0.245	0.060	10
RATIO D/E	0.390	0.152	10
	-0.120	0.014	10

Note: The significance (*) of the correlations were determined at $p \leq 0.05$ level using a tabulated set of significance limits.

TABLE IV.1.6
CORRELATION COEFFICIENTS FOR SELECTED CHARACTERS, IRRESPECTIVE OF SITE

	Corr.(r)	r^2	d.f.
X:Oil Yield Y:%Dry Matter	-0.200 n.s.	0.040	27
X:Oil Yield Y:Glands/mm	0.160 n.s.	0.026	27
X:Dry Matter Y:Glands/mm	-0.050 n.s.	0.002	27

Note: The significance of the correlations were determined at $p \leq 0.05$ level using a tabulated set of significance limits.

1.2 SELECTION OF CLONES

1.2a ORGANOLEPTIC ASSESSMENT AND PERCENTAGE OIL YIELD

At Great Lake, Elephant Pass and Paradise Plains a large population of plants was available. At these three sites the following procedure was employed to select suitable plants for cloning. An initial selection of several plants was made in the field by crushing leaves and noting whether they contained an oil with pleasant organoleptic properties.

Samples were collected from these plants for steam distillation and oil evaluation. The final decision on which particular plant to use was based on a balance between a desirable, pleasant odour, its persistence and as high an oil yield as possible, together with ease of propagation. Table IV.1.7 shows the last stages of this process which resulted in EP 26, PP 6 and GL 42 being chosen as clonal material.

At the remaining three sites, where the population was relatively sparse, the clone was chosen on the basis of observations made in the field. The leaves of plants that looked vigorous were crushed between the fingers, and the choice was made on the basis of initial odour impact.

The six clones then, were called GL (Great Lake 42), PP (Paradise Plains 6), EP (Elephant Pass 26), MW (Mt. Wellington, near

Fern Tree), BU (Buckland) and EN (Eaglehawk Neck).

TABLE IV.1.7

SELECTION OF CLONES FROM

ELEPHANT PASS, GREAT LAKE AND PARADISE PLAINS

(note: persistence was recorded as 0 if the odour was not persistent; * to *** for increasing persistence; percentage oil yield is given on a dry matter basis)

	Odour	Persistence	% Oil Yield
ELEPHANT PASS			
7	weak	0	0.305
9	weak	0	0.200
12	tomato, spicey	0	0.314
15	faint, citrus-like	0	0.310
26	fairy strong, spicey, biting, citrus-like	**	0.346
27	robust, caramel-like, spicey	***	0.121
29	biting, unpleasant herby	**	0.171
37	faint	*	0.345
41	strong, thick, herby	0	0.545
44	like 41	**	0.246
46	faint, unpleasant	**	0.591
49	like 26, weak	0	0.080
GREAT LAKE			
11	fresh, not spicey	*	0.181
24	strong, 'typical'	**	0.167
25	fresh, spicey	*	0.208
27	biting, spicey, tomato, fresh	*	0.162
42	strong, tomato, fresh heavier than others	**	0.222
PARADISE PLAINS			
6	distinct, tomato, spicey	**	0.461
7	faint	**	0.281
9	mouth-watering, thin	*	0.237
10	spicey	**	0.293
12	typical PP	*	0.332
15	similar to 6	*	0.863
18	typical PP, heavy	**	0.288
22	raw, strong	**	0.470
27	spicey	**	0.231
34	faintly spicey, papery	*	0.463
39	spicey but thin	*	0.249
45	faint	*	0.449

1.3 ASPECTS OF CLONAL CULTURE

1.3a PRELIMINARY PROPAGATION TRIAL

A small-scale trial was performed to assess the germination rate of Olearia seeds. Five replications of 20 seeds were placed on Whatman No. 1 filter paper, in Petri dishes, and moistened with 3 ml of distilled water. The dishes were held at 22°C with 16 hours light, 8 hours dark. (Forsyth C. and Van Staden J., 1983)

After a period of six weeks, germination did not exceed 5%, with 15% in only one of the replications. Clearly, seed germination occurs rarely under these circumstances.

The work by Forsyth and Van Staden was conducted on the essential oil bearing Composite *Tagetes minuta* L. This species also has single-seeded achenes similar to, but longer, than those of Olearia. They found that the achenes have both a temperature and a light requirement for germination, with optimal germination occurring at 25°C under white light. Seeds of *Olearia* sp. have a more rigid germination control system, and its optimal requirements for germination are unknown.

On the other hand, results from cutting trials were excellent, with percentage strike rates for the clones selected for further investigation as shown in Table IV.1.8 below. Tip cuttings were taken throughout the spring-summer growing season, when sites were visited for the collection of morphological measurement data. The cuttings were placed in punnets (12 per punnet), of a mixture of equal parts sand and peat. The total number of cuttings of each clone was always 60 or more. No rooting hormones were applied, and the growing medium was not sterilised, since previous work has shown that the strike rate actually decreases when these procedures are applied (Dragar V.A., 1984).

TABLE IV.1.8
STEM CUTTING STRIKE RATES FOR SELECTED CLONES

CLONE	%	CLONE	%	CLONE	%
PP 6	89	GL 11	25	EP 7	94
7	18	24	25	9	58
9	86	25	21	12	83
10	44	27	40	15	58
12	42	32	25	26	98
15	15	33	21	27	100
18	35	42	89	29	100
22	10	49	24	37	97
27	36			41	81
34	8			44	64
39	99			46	69
45	40			49	58

The strike rates for the other three clones were 97%, 95% and 67% for EN, MW and BU respectively.

These results were used in combination with the percentage oil yield and organoleptic assessments to identify clones suitable for further trials. The final clones selected were GL 42, PP 6, EP 26, MW, BU, and EN.

2. CHEMICAL COMPOSITION OF OLEARIA PHLOGOPAPPA ESSENTIAL OILS

2.1 INTRODUCTION

The essential oils of the six Olearia clones have individual odour characters. Each oil has an odour which is distinctly different from the others. It is well known that even minor differences in key components of an essential oil can confer large variations in overall odour properties. The MW, PP and GL oils all have interesting, spicey notes. However, of these MW is the most outstanding. The identities of the components responsible for this unique desirable trait were therefore sought.

The purpose of essential oil analysis was multi-faceted. Firstly, the oils from the six clones can be qualitatively compared by gas chromatography or high performance liquid chromatography. In addition, gas chromatography/mass spectral studies can provide information about the actual components present in the oils.

By semi-preparative analysis of an essential oil, an odour profile can be obtained, evaluating each fraction as it is obtained from the column. In this way, the interesting components which give spicey and exotic fruity notes can be singled out in the chromatogram, and their further investigation can follow.

Many methods of isolating the desired components of essential oils have been used. For instance, a continuous counter-current separator with 680 cells was used to isolate the 'honey-note' of rose oil. This method was chosen by the author because it is a very mild separation method, which can be used to separate a number of components when used with various solvent systems. (Kovats E., 1987). However, the resolving power for a complex mixture is limited, which leads to fractions that still contain several components.

The techniques available for the separation of the Olearia oil into fractions were high performance liquid chromatography (hplc) and gas chromatography (gc).

2.2 SEPARATION STRATEGIES

Essential oils are complex mixtures. Therefore, the preparative separation strategy should seek to fractionate the whole oil into classes or groups of components relatively quickly and economically, using crude separations. Then, the individual components can be isolated by more refined methods. Ideally, the initial separation of the essential oil into workable fractions should be as 'mild' as possible. The aim is to avoid the formation of artifacts by the separation procedure through dehydration, oxidation or isomerisation, of the sample.

A silica gel column has been frequently used for separation of essential oils (Scheffer J.J.C. *et al*., 1977, Kubeczka K.-H., 1973). Such a column is useful in that large amounts of material can be processed very rapidly (10-15 minutes), with a simple column, using a variety of solvents as the mobile phase.

The major disadvantage of silica gel separation is that there is a high risk of chemical reactions being catalysed during the separation process. However, essential oils can be separated into classes of compounds such as hydrocarbons, esters, aldehydes, ketones and alcohols, which facilitates subsequent separation by gc or hplc.

2.2a GAS CHROMATOGRAPHY

Gas chromatography (gc) was considered in the first instance for preparative work. However, this isolation procedure necessitates heat treatment of the column effluent, which can often lead to chemical changes of some oil components. Thermally labile components will undergo changes as they pass through any type of column. Thus, an altered odour impression is obtained when using a heated gc exit port. This is an obvious disadvantage if one is attempting to determine an odour profile, or isolate a particular note from the oil.

High chemical reactivity can be overcome with the use of fused silica analytical columns, since they are more inert. However, for preparative purposes a larger, packed glass column is necessary, which does not share these attributes.

The organoleptic evaluation of an essential oil through the use of a heated gc exit port, as well as other gc techniques using capillary columns is reviewed in the literature (Motto M.G., 1987).

2.2b HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Strack D. *et al* ., 1980 report the use of reversed phase hplc analysis in characterisation of essential oils. They were able to resolve complex mixtures of sesquiterpenes and oxygenated volatile constituents with a water/acetonitrile solvent system. The separations achieved were comparable to that of gas chromatographic analysis.

The range of hplc columns available for the Olearia oil separations included both reversed phase and normal phase types. The resolution of a mixture of closely related substances requires the careful selection of column and mobile phase. During the course of the separation work, both types of column were used, since, in general, the non-polar components are more readily separated on the reversed phase column, and the polar ones on silica gel. The reversed phase column was used with acetonitrile as the mobile phase for the fractionation of MW oil, and the subsequent characterisation of the aroma of each fraction. In this way the components with spicey aromas were recognised.

Since most of the separation work was done by hplc, it was obligatory to perform analytical runs on all of the Olearia oils, using this method. The chromatograms that were obtained were compared to those obtained by gc in order to assist in the location of components of interest. The differences between the oils were also evident from this data.

The choice of detector is also very important. The ultra-violet (uv) detector system is most commonly used with hplc experiments. It was not possible to scan the whole spectrum for each elution peak, as is possible with a diode array detector. Since components have differing absorption maxima, quantitative analysis of components was not possible. The extreme example of this occurs when there is virtually no absorption at the chosen wavelength. The uv spectrum would have been useful, however, in the identification of components.

Under these circumstances, an alternative detector can be used.

The refractive index (ri) detector measures variation of refractive index in the column effluent. Since refractive index changes by about 1×10^{-4} for each degree change in temperature, it is necessary to control the ambient temperature of the detector cell very carefully. As an example, Komae H. and Hayashi N. (1975), used both uv and ri detection to analyse the essential oils fo two *Lindera* species.

The ri detector is a universal detector in that all components will be detected, as long as their refractive indices are not identical with that of the mobile phase. One major obstacle to using ri detection is the long equilibration times necessary when either flow rate or solvent is altered. This makes ri detectors impractical for gradient elution.

2.2c METHODS OF OIL COMPONENT IDENTIFICATION

The course of the analytical work began with the analysis of all six Olearia oils by gas chromatography and mass spectroscopy. This provided a framework from which to target components for elucidation of their structure. From the gc/ms data, relatively few components of the oil could be identified immediately. In fact, the major compounds which make up the bulk of the oil were initially unidentified.

A combination of methods was used for the identification of the components of the oils. These were mass spectroscopy (ms), Fourier transform infra-red spectroscopy (Ftir) and nuclear magnetic resonance spectroscopy (nmr) techniques.

As an adjunct to the ms work, Ftir analyses provided some further information confirming some of the proposed components. The low sensitivity of Ftir analysis required larger volumes of samples to be injected. This caused a loss of resolution. However, the ability to distinguish functional group moieties proved useful.

Nmr analysis was used only for the structural elucidation of isolated components.

2.2d SAMPLE STORAGE EXPERIMENT

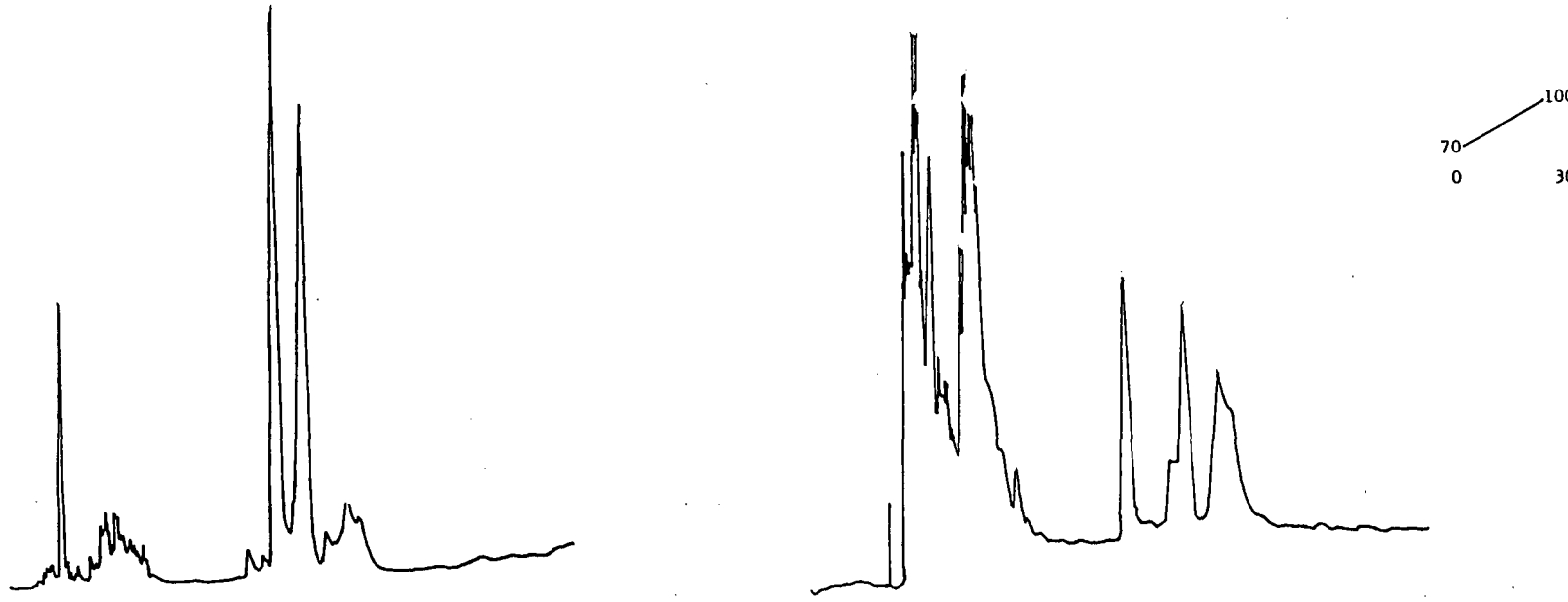
The isolation and identification of essential oil components is a time consuming procedure, which often necessitates the storage of samples over long periods of time. It was suspected that component modifications may occur in oil samples pre-mixed with tetrahydrofuran for injection onto the hplc column. Thus, a comparison of 'old' sample (about six weeks old), with a freshly prepared one, was made. The two samples were run in acetonitrile/water, 70% to 100% in 30 minutes. The resulting traces are shown in Figure IV.2.1. It was obvious that the sample degraded with time, with a decrease in unsaturated sesquiterpene hydrocarbon type components occurring with a corresponding increase in polar oxygenated compounds. In other words, oxidation of the sample was occurring probably due to the very ready formation of peroxides from tetrahydrofuran.

Thus, for this type of work it was vital that whole oil samples be freshly prepared regularly, and that collected fractions be examined by nmr or other further analytical techniques as quickly as possible to prevent the build-up of impurities.

FIGURE IV.2.1

SAMPLE STORAGE EXPERIMENT

HPLC TRACES OF FRESH AND AGED OIL SAMPLES



New Oil Solution

Old Oil Solution

2.3 PREPARATIVE SEPARATIONS

2.3a SILICA GEL PRE-FRACTIONATION

Olearia essential oil is a very complex mixture of diverse components, and their separation is not straightforward. In an effort to improve the separation, a pre-fractionation step was introduced into the method along the lines of that employed by Scheffer J.J.C. *et al.*, (1977).

All six Olearia clone oils were separated into three fractions using a simple silica gel column.

Silica gel (12 g) was dry packed into a small sintered glass funnel. The essential oil was introduced onto the column as a hexane solution (20% v/v approx.). The three fractions were eluted using:

Fraction A	100 ml	hexane
Fraction B	100 ml	4% THF in hexane
Fraction C	60 ml	33% THF in hexane

Table IV.2.1 shows the relative percentage weight of each of the fractions recovered from the six oils.

TABLE IV.2.1

RELATIVE PERCENTAGE WEIGHTS OF THREE FRACTIONS RECOVERED FROM A SILICA GEL COLUMN FOR SIX OLEARIA OILS

	GL	MW	EN	PP	EP	BU
Sample Date	8/87	8/87	8/87	8/87	6/88	7/88
Fraction A	48.26	46.01	65.27	20.53	31.58	61.75
Fraction B	23.94	29.14	14.12	46.39	32.33	19.65
Fraction C	27.80	24.85	20.61	33.08	36.09	18.60

The components found in each fraction are related by polarity. Thus, fraction A contains the hydrocarbons, fraction B the oxygenated compounds, ethers, esters and aldehydes, whilst the alcohols and other more polar compounds are concentrated in the last fraction.

TABLE IV.2.2

COMPILATION OF COMPOUNDS FOUND IN SILICA GEL FRACTIONS FROM SIX CLONAL OIL SAMPLES

Fraction A	Fraction B	Fraction C
3.83 not BU		
4.52 not BU		
4.56 PP		
4.59 QL EP BU		
5.23 MW QL		
5.51 MW QL PP EP		
5.59 EP BU		
5.70 MW EP EN		
5.74 ALL		
6.25 QL	6.25 EP	
6.46 MW QL PP EP		
	7.35 ALL	
	7.41 MW QL	
	9.15 PP	
		9.46 PP
	11.70 PP BU EN	
	11.85 MW QL PP	
	12.00 MW BU EN	
12.27 PP		
	12.44 EN	
12.70 EP		
13.09 BU		
13.18 EP EN		
13.39 ALL		
13.48 EN		
	13.79 MW BU	
	13.95 QL EN	
	14.00 MW QL BU EN	
14.18 ALL		
14.30 PP	14.30 MW	
14.63 MW PP EP EN		
14.79 MW PP EP EN		
15.00 MW PP EN EN		
15.60 not PP		
15.76 not MW		
15.91 ALL		
16.00 MW QL EN		
16.18 ALL		
16.29 QL PP EN		
	16.37 EP PP QL	16.37 EN BU
16.47 ALL		
16.55 PP QL EP	16.55 BU EN	
16.63 PP QL	16.63 MW	
16.65 QL PP EP EN		
	16.75 MW QL BU	
	16.85 EP BU	16.85 EN
16.97 QL PP BU		
	17.05 MW BU	
	17.16 MW	
	17.35 PP EP	
	17.48 MW EP EN	
17.56 QL EN		
	17.68 not EN	
	17.74 QL PP	
17.82 EP MW	17.82 BU EN	
	17.95 MW PP EP BU	
		18.03 MW PP
	18.20 EP PP	18.20 MW QL EN BU
	18.36 EN	
	18.43 EP	18.43 MW
	18.55 PP EN	18.55 QL BU
	18.64 BU	18.64 QL PP
	18.73 MW PP EP EN	18.73 BU
	18.86 QL EP	
	18.95 MW EP BU EN	
	19.06 QL PP EP EN	
	19.13 MW PP BU EN	
		19.21 EN
		19.34 MW EN
	19.36 MW PP EP	
		19.45 not PP
	19.54 MW QLL EP	
		19.63 MW PP EP EN
	19.76 QL PP EN	
		20.02 EN
		20.16 MW EN
	21.36 EN	
	22.04 EN	
	22.94 ALL	
23.05 not QL		
24.30 MW		
	24.94 MW EP BU	

Each of the fractions was examined using the standard gc methodology. Table IV.2.2 presents a compilation of the compounds in all of the six oils, found in each fraction. The components are designated by their gc retention times (minutes), and the oil in which they were found is indicated by the clonal code (MW, PP, EP, GL, EN or BU). Some components seem to appear in different fractions in different oil. The fact that, for instance, the peak at 14.30 min eluted in Fraction A from PP oil and in Fraction B in MW oil, suggests that these are different compounds in each case, having the same retention time. The table lists numerous similar examples.

The fractionation process also shows that hydrocarbons occur throughout the retention time range, comprising both monoterpenoid and sesquiterpenoid components. The oxygenated components do not occur very early in the chromatogram, but persist, with greater frequency than the hydrocarbons, to the end. The components in the final fraction begin to elute from the column as late as 9.5 min and constitute less than one quarter of the overall number of components.

Some peaks present in the whole oil were not found in the resultant fractions. A list of these is given in Table IV.2.3.

TABLE IV.2.3

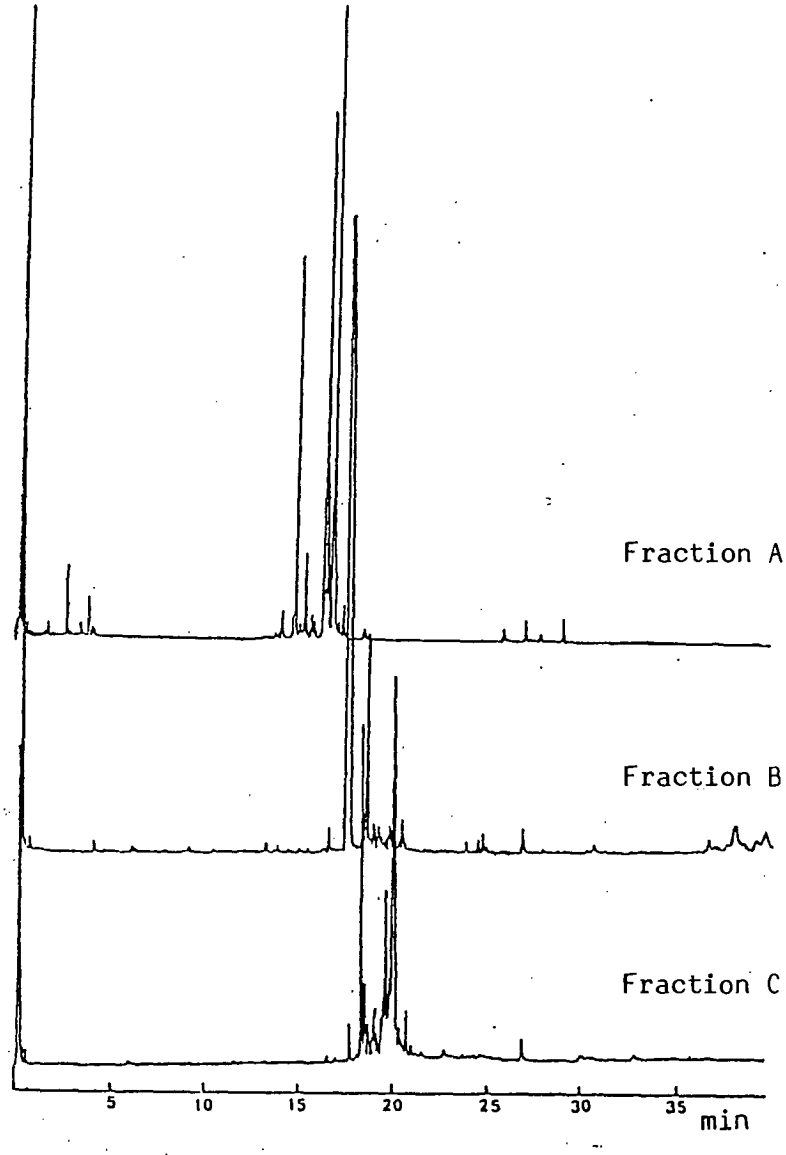
COMPONENTS PRESENT IN WHOLE OIL SAMPLES

NOT FOUND IN SILICA GEL FRACTIONS

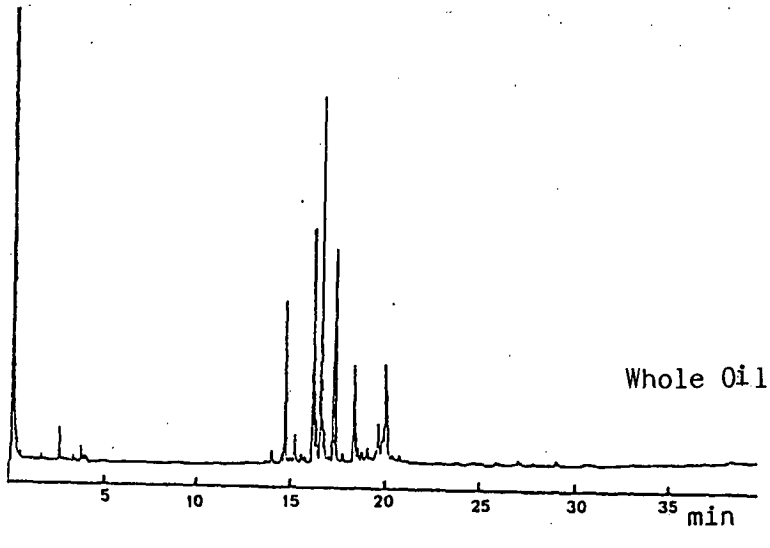
PP	GL	MW	EN	BU	EP
4.61					
9.15					
	12.28				
			13.09		
			14.26		
	14.74				
15.33	15.33		15.33	15.33	
		15.76			
					16.08
				16.29	
	16.86	16.86			
	18.36	18.36		18.36	
18.87					
	18.96				
19.92	19.92				

From the above table, it seems that certain components tend to

FIGURE IV.2.2



Gas chromatograms of silica gel fractions and MW whole oil.



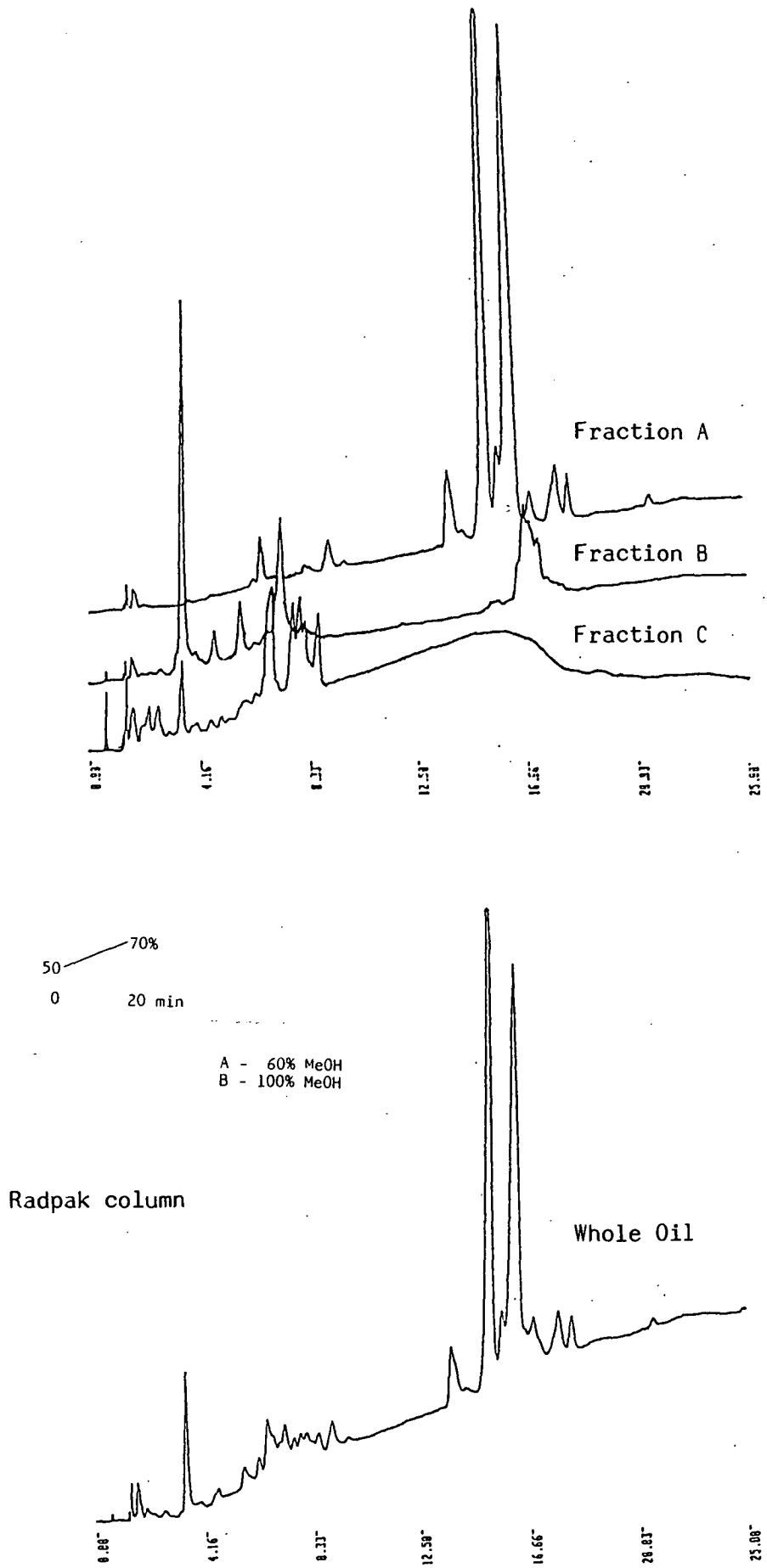


FIGURE IV.2.3

Hplc chromatograms of silica gel fractions and MW Whole oil.

be retained by the silica gel during the elution procedure. Thus, they do not appear in the chromatograms. The multiple occurrence of some peaks in Table IV.2.3 suggests that the retention of these components occurred during the elution of all the oils, to some extent. An alternative explanation might be that they were converted, during chromatography, into other components already present in the oil.

Careful inspection of the gc traces of the three fractions compared to the whole oil revealed that the passage of the oil through the silica gel did not cause the formation of any components that were not present in the original sample. That is, no artifacts were detected.

An example of the separations achieved is given in Figure IV.2.2, where gas chromatograms are presented of the whole MW oil, and the three fractions derived from it. Figure IV.2.3 shows the hplc traces from the same samples.

The three fractions had distinctly different odours. Fraction A was fruity and floral. Fraction B was more spicey and characteristically Olearia-like, while the last fraction was was musty and had little impact.

The hydrocarbons are collected in Fraction A, and include most of the monoterpenes visible in the oil, with the exception of a few oxygenated compounds in Fraction B.

Therefore, the use of the silica gel fractionation method proved extremely useful in the initial separation of these compounds. The method is both quick and simple, and provides an excellent base for further separation work.

2.3b PREPARATIVE GAS CHROMATOGRAPHY

Initially, an investigation was made into the possible application of preparative gas chromatography for the separation of Olearia oil samples.

Preparative gas chromatographic (gc) analyses of essential oil samples were performed using a Pye Unicam Series 104 Gas Chromatograph. The glass column and operating conditions used are described in Materials and Methods.

The injected sample was allowed to elute from the column under these conditions. The majority then passed through the exit port, where it was collected in a glass U-tube, immersed in and ice/salt freezing mixture.

The collected samples were examined by analytical gc using the standard methodology (Materials and Methods). Only limited success was achieved with the gc isolation of oil components, with no pure components being obtained. The method proved to be tedious, with only small injection volumes giving adequate resolution. It is well known that many of the components of essential oils are heat labile, and degradation products may have contributed to peak broadening.

The primary interest was to isolate the components of the oil which contribute most to the characteristic odour of the product, as well as its major components. The eluent from the column was monitored by means of a heated 'sniffing port', so the odours of the successive compounds could be recorded. A few of the odour impressions observed from BU oil are shown in Figure IV.2.4 (b).

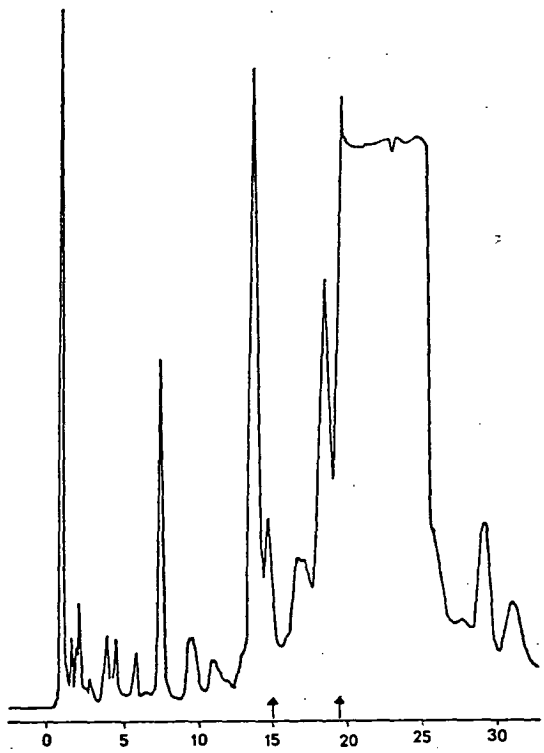
An odour note that may be basically inappropriate to an odourant may be assigned to a gc peak for a variety of causes. In the first instance, the odour of a strongly odorous peak which is next to elute may be building up in advance of a visible detector response, and may be assigned to the preceeding peak.

In addition, the compounds which exit the column may be changed from those in the original oil sample, either by their passage through the column, or through the instantaneous reactions occurring in the injector. These latter alterations would not be detected.

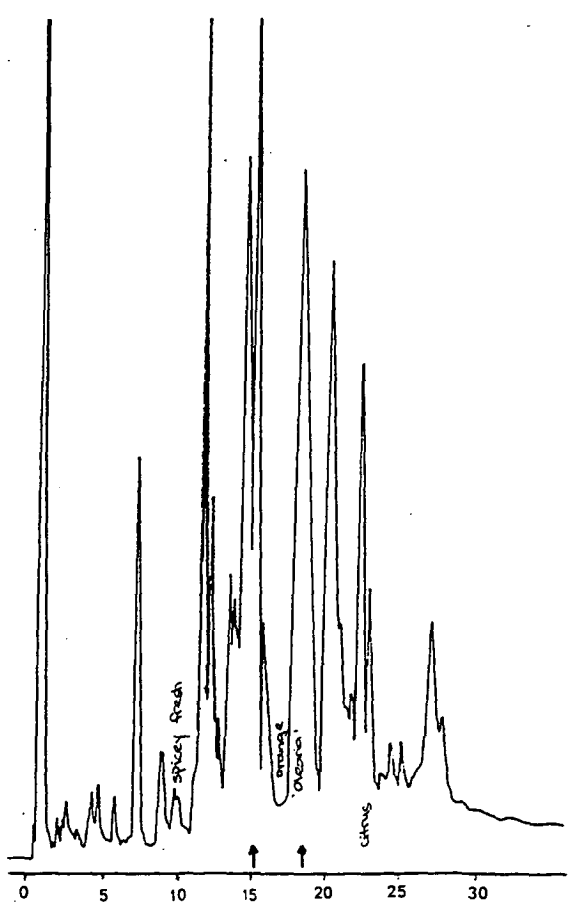
The resolution achieved is shown in Figure IV.2.4. The injection volume was 1µl of neat BU oil for this run. The components that were of interest lay between the arrows, as shown.

FIGURE IV.2.4

PREPARATIVE GAS CHROMATOGRAPHIC TRACES OF OLEARIA OIL



(b) 10µl BU



(a) 1µl BU

When the volume of oil injected was increased to 10µl, detector overloading occurred and some loss of resolution resulting in traces like that in Figure IV.2.4 (b). In particular, the region of interest in the chromatogram appears to be overloaded, which could not be resolved with changes in flow rate or temperature program.

The major components of Olearia oil constitute some 30%, say, of the oil. With 1µl injections of neat oil, some 0.3 mg can potentially be collected. This would mean that numerous repeated runs would have been required to accumulate sufficient material for identification work. Therefore, another method of separation was sought.

2.4 ANALYTICAL DETERMINATIONS

2.4a GAS CHROMATOGRAPHY/MASS SPECTROSCOPY

Using the method detailed in Materials and Methods, samples from Bushy Park, (August '87), were analysed by gas chromatography/mass spectroscopy (gc/ms), and compared with library spectra. The gc traces are shown for the GL, EP, EN, BU, PP and MW clones in Figure IV.2.5. Figure IV.2.6 contains the gc/ms total ion count (t.i.c) traces for these same clones, respectively. Among the six clones, the following were identified:

1. α-pinene
2. β-pinene
3. 1,8-cineole
4. linalool
5. α-terpineol
6. caryophyllene
7. germacrene-D
8. bicyclogermacrene
9. elemol
10. spathulenol
11. γ-eudesmol
12. β-eudesmol
13. α-eudesmol

These numbers appear on the chomatograms as peak labels.

The major peaks are germacrene-D and bicyclogermacrene, in general. However, in some oils, the eudesmols comprise some 25% of the oil. In PP all three isomers are present, whereas in EP only β-eudesmol is present in proportions reaching 35%. Another major difference in the oils involves their caryophyllene content. Although this compound is present in oil from all six clones, in GL it represents some 37% of the total volatiles. In the other five clones, there is a maximum of only 6% caryophyllene.

A list of components and the oils in which they occur is given below in Table IV.2.4. This list includes some unidentified components, which were also detected by gc/ms and which have been labelled using letters of the alphabet. The presence of a component is indicated by '+'.

FIGURE IV.2.5

GAS CHROMATOGRAMS FOR SIX OLEARIA CLONES

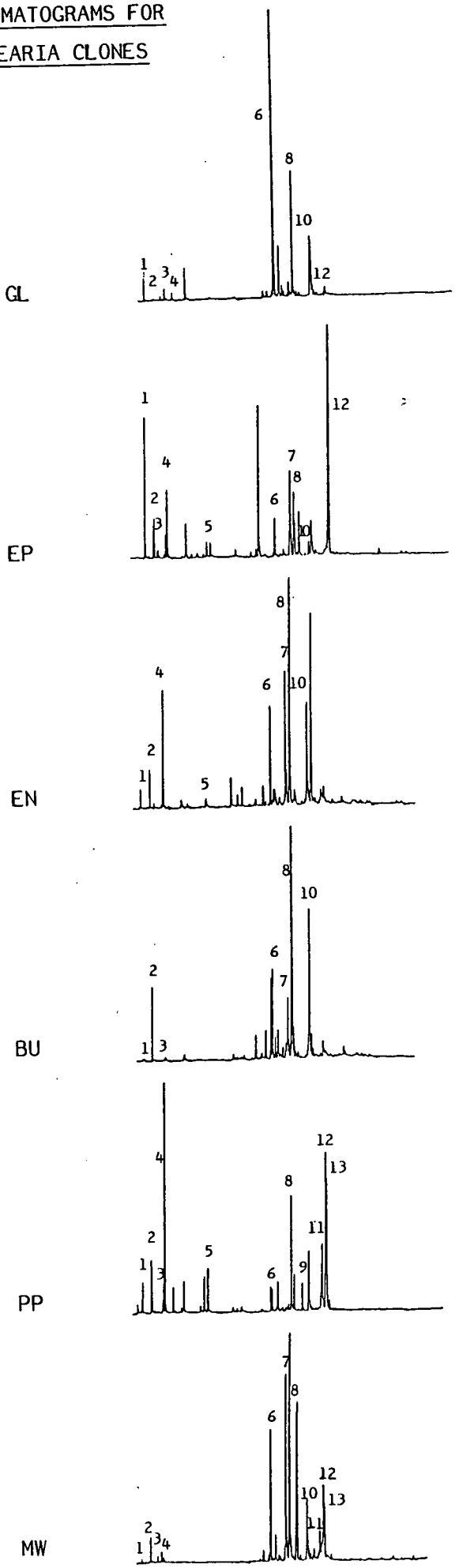


FIGURE IV.2.6

GC/MS TOTAL ION COUNT TRACES FOR SIX OLEARIA CLONES

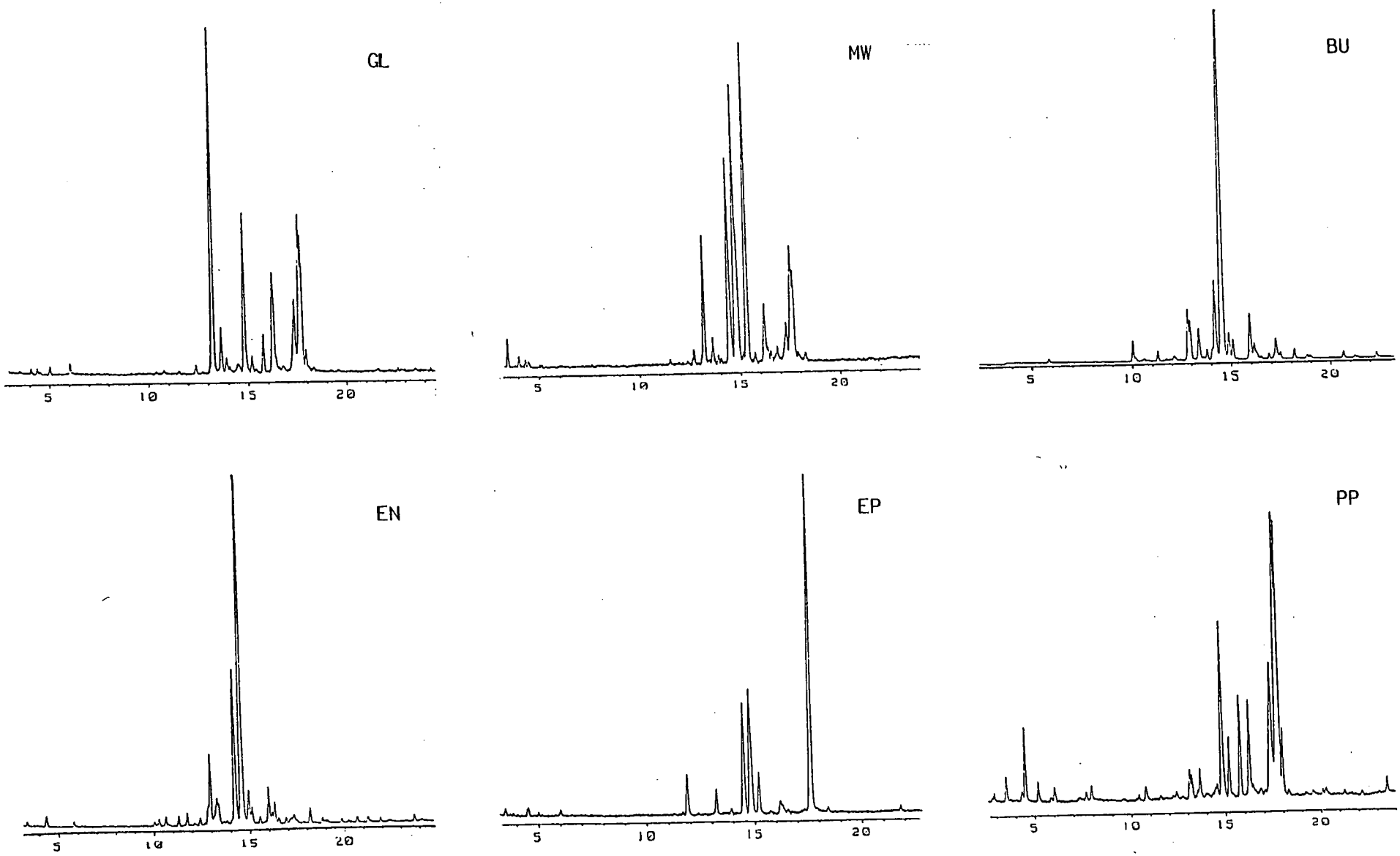


TABLE IV.2.4
CLONAL ESSENTIAL OIL COMPONENTS

	PP	GL	MW	EP	EN	BU
α-pinene	+		+	+	+	
β-pinene	+	+	+	+	+	
1,8-cineole	+	+		+		
linalool	+	+		+	+	+
α-terpineol	+					
a BU 10.025						+
b BU 11.273						+
c MW 13.622	+	+	+		+	+
caryophyllene	+	+	+	+	+	+
germacrene-D	+	+	+	+	+	
bicyclogermacrene	+	+	+	+	+	+
δ-cadinene						+
e MW 14.740		+	+	+	+	+
f MW 14.953	+		+			
g MW 15.152	+		+		+	
h MW 15.294			+			
kessane			+			
liguloxide			+			
caryophyllene oxide			+			
i PP 15.232	+					
j GL 15.189		+				
k MW 15.164			+			
l EP 15.203				+		
m EN 15.129					+	+
elemol by ms 15.803	+					
n MW 15.734		+	+			
o EP 15.742				+	+	
p MW 16.885	+		+			
spathulenol	+	+	+		+	+
γ-eudesmol	+	+	+			
β-eudesmol	+	+	+	+		
α-eudesmol	+	+				
q MW 18.264	+	+				
r			+			+
s MW 15.00	+		+			

NOTES: unknowns are labelled a to u.
q is an elemol-type compound
r occurs adjacent to bicyclogermacrene
s is a sesquiterpene hydrocarbon

From the above table, it is evident that the composition of each of the oils is distinctly different from the others. It appears that only two compounds occur ubiquitously, namely bicyclogermacrene and caryophyllene. In MW and BU the bicyclogermacrene peak is actually a mixture with another

sesquiterpene which has a diagnostic peak in its mass spectrum at m/e 119. This is a major peak and may be zingiberene.

Some components occur in all oils except one. For instance, linalool is seen in all except MW oil, and β-pinene and germacrene-D are absent only from BU oil. The component assigned as 'c' is not present in EP oil, but occurs in all other instances, while compound 'd' is absent only from PP oil.

The eudesmols are also widespread, however, in two cases they are associated with a peak that elutes from the column immediately after. This peak 'q', has an elemol-type mass spectrum, and occurs in PP and GL oils.

There are numerous instances where a component occurs only in one oil, including elemol, which is seen only in PP oil. At a similar retention time, in GL and MW, compound 'n' occurs, with a base peak at m/e 44 and a molecular weight of 188, compared to a base peak at m/e 59 and a molecular weight of 204 for elemol. In EP and EN, at a similar retention time, a third compound, designated 'o', is observed with a base peak at m/e 44 and a molecular weight of 118.

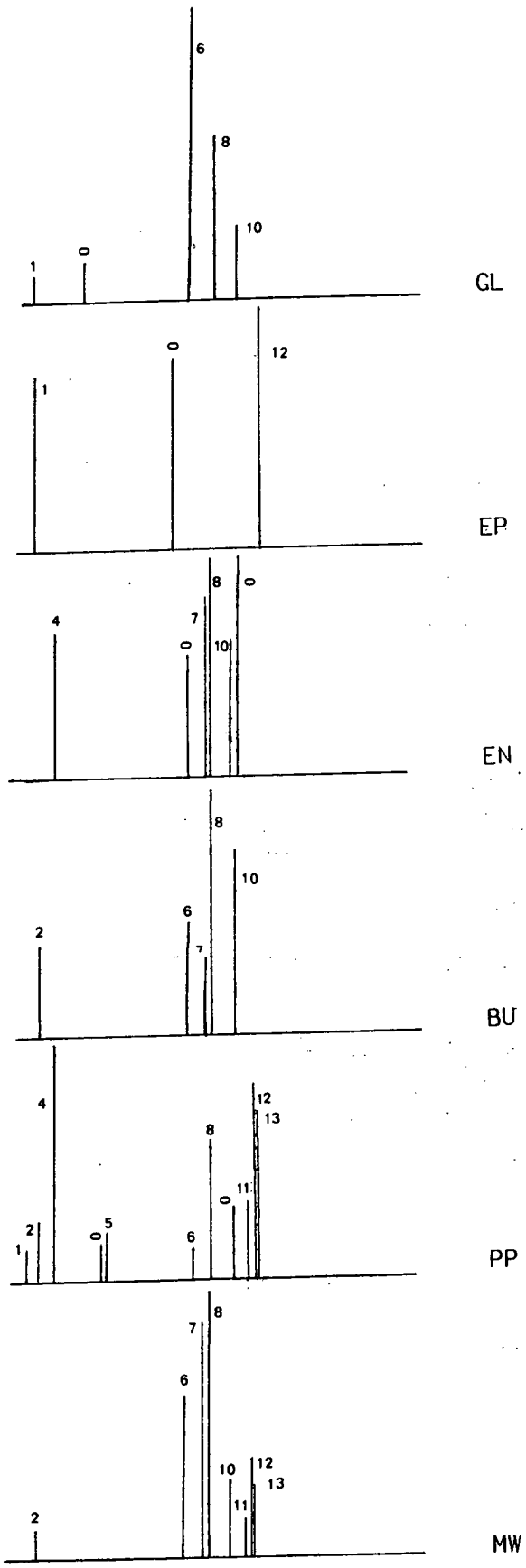
Other compounds which occur only in one oil include α-terpineol, elemol and i (at 15.2 minutes) in PP, two peaks, (a and b), around 10 and 11 minutes and δ-cadinene in BU, four sesquiterpene hydrocarbons, (h, k, kessane and liguloxide), around 15 minutes, and caryophyllene oxide in MW, l in EP (at 15.2 minutes) and j (at 15.2 minutes) in GL. In fact, the only oil which does not bear a unique component is that from EN. The components caryophyllene oxide, kessane and liguloxide were only unambiguously identified in MW oil, but there is a possibility that they occur in small amounts in the other oils.

By using Figure IV.2.7, an overall impression of the oils and their complexities can be gained. This figure gives a sketch of the major peaks and their relative intensities in their simplest form. The peaks that have been identified have been labelled with the appropriate peak number, and the unknowns are labelled ○.

Of the six oils, MW and PP are the most complex mixtures, and this is reflected in the odour of these oils. Only PP has a large proportion of monoterpenes. In all the other oils, the prevalent components are sesquiterpenes. The percentage of monoterpenes present in each of the oils was calculated using the chromatograms

FIGURE IV.2.7

MAJOR ESSENTIAL OIL COMPONENTS



o = unidentified

of the oils from the '88 harvest. These are presented in Table IV.2.5.

TABLE IV.2.5
PERCENTAGE MONOTERPENES IN '88 HARVEST OILS
SOLVENT EXTRACTION SAMPLES

CLONE	MW	GL	PP	EP	EN	BU
OUSE	7.90	6.28	10.92	5.60	6.55*	9.76
BUSHY PARK	5.83	5.94	12.74	5.80	12.80	2.08*

In general, all the oils have a low monoterpene content, with the highest being 12.8%. The values marked * are uncharacteristically low, and may be due to the handling of samples. (Results of gc analyses are not reliable when the concentration of oil is low, and the internal standard consistutes over 30% of the sample).

EN and BU are the 'simplest' oils, and it is perhaps pertinent that these two clones are large leaf types, whereas PP, GL and MW are small leaf varieties. The EN clone has oil which is very similar in composition to BU oil, and tends to be a 'simple' large leaf type. Conversely, GL is a small leaf clone, and although the sketch of its major peaks is fairly simple, its odour and leaf type would suggest that it could be grouped with MW and PP.

The PP clone contains the most monoterpenes of the small leaf clones, and EP, due to its high levels of β-eudesmol, has the most sesquiterpenes of the large leaf types.

Comparisons such as the ones made above are useful for the establishment of the identity of any given Olearia oil. Each oil is sufficiently different in terms of its constituent compounds and their relative concentrations, that they can indeed be regarded as distinct varieties, or can at least be placed into two discrete categories.

Further analytical work was carried out using Fourier transform infra-red spectroscopy. In conjunction with mass spectroscopic data, the identity of several compounds was confirmed.

2.4b FOURIER TRANSFORM INFRA-RED SPECTROSCOPY

Samples of the essential oils from the six clones were analysed by gas phase absorption as detailed in Materials and Methods. The figures presented show only those compounds which were identified, while descriptions of interesting facets of components which remained unidentified, are given latter in the text.

A major limitation of the gc/Ftir method was that only the principal components could be studied. This was due to the limited number of transients that could be acquired for each peak. Therefore, only several spectra could be obtained for each oil.

I. IDENTIFIED COMPONENTS

SPATHULENOL

Figure IV.2.8 shows the Ftir spectrum and structure of spathulenol. The presence of an alcohol moiety is shown by the absorptions at 3600 cm^{-1} (δ(O-H)) and 1091 cm^{-1} (δ(C-O)). The exocyclic double bond is indicated by absorptions at 3090, 1636 and 891 cm^{-1}. This component is was shown to be present by Ftir in MW and GL oils.

ELEMOL

Figure IV.2.9 was obtained from PP oil. The ms data on this component suggested that it was either elemol or hedycaryol with the formula $C_{15}H_{26}O$. The Ftir spectrum is only consistent with the elemol structure (Figure IV.2.9), as the two absorptions at 893 cm^{-1} and 910 cm^{-1} show the presence of exocyclic terminal double bonds. The δ(O-H) frequency at 3645 cm^{-1} indicates a tertiary alcohol.

β-EUDESMOL

Another compound to be identified by Ftir was β-eudesmol. The spectrum and structure are given in Figure IV.2.10. The absorption spectrum is consistent with the suggested structure due to the

FIGURE IV.2.8

FTIR SPECTRUM OF SPATHULENOL

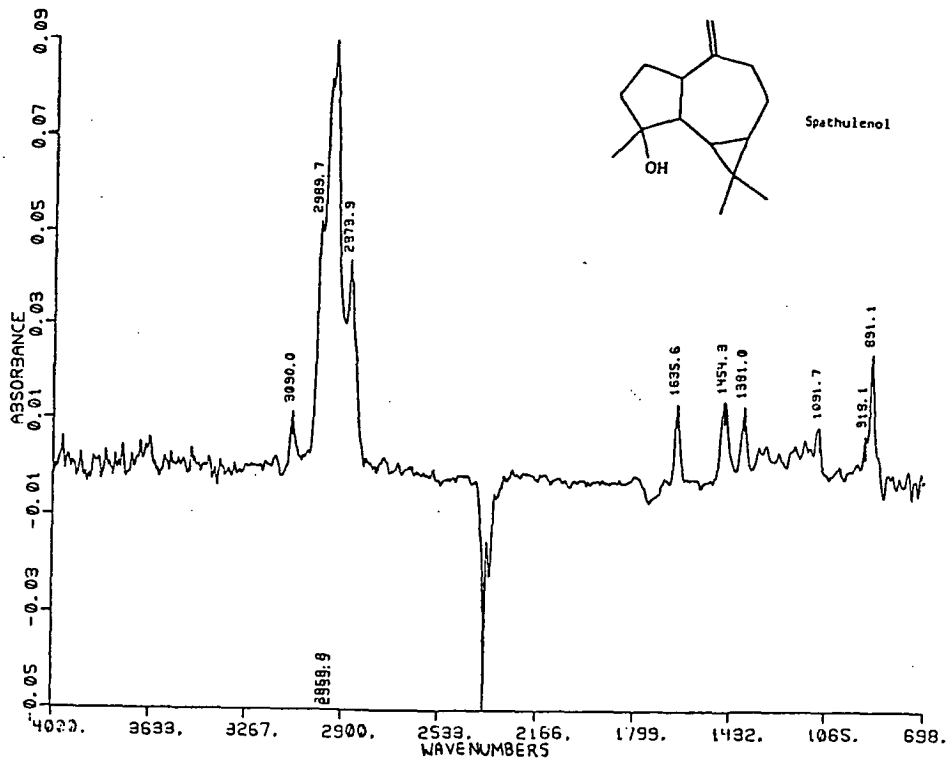


FIGURE IV.2.9

FTIR SPECTRUM OF ELEMOL

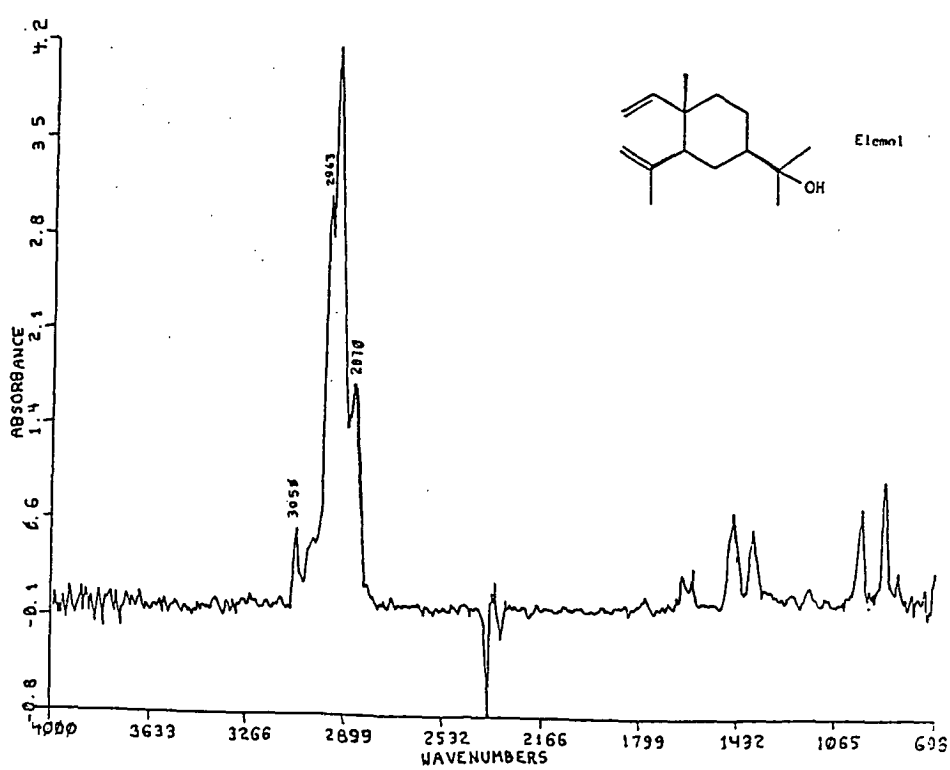


FIGURE IV.2.10

FTIR SPECTRUM OF β-EUDESMOL

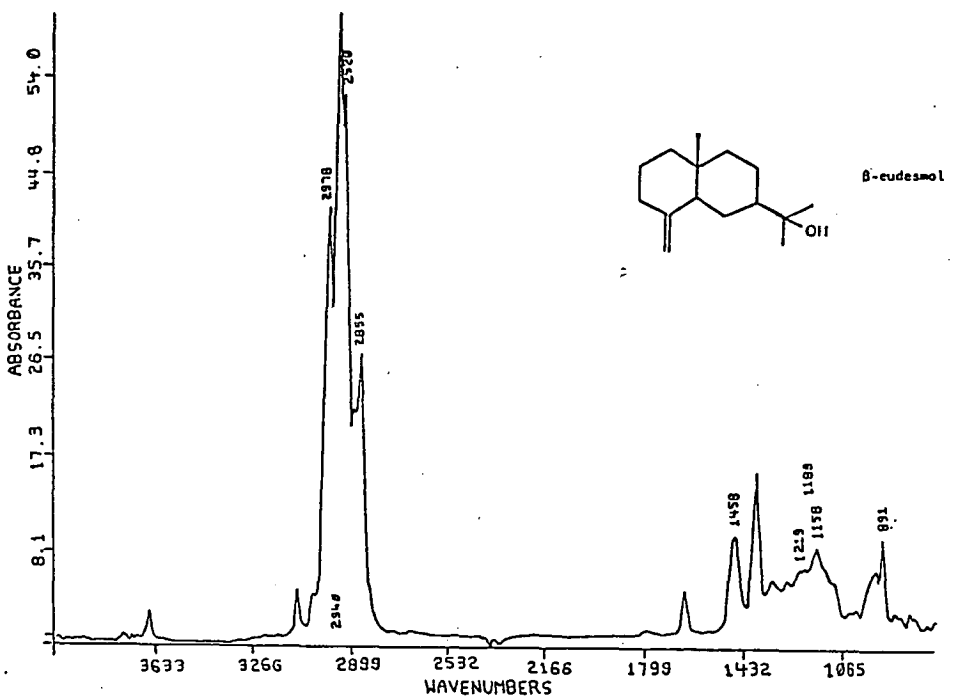
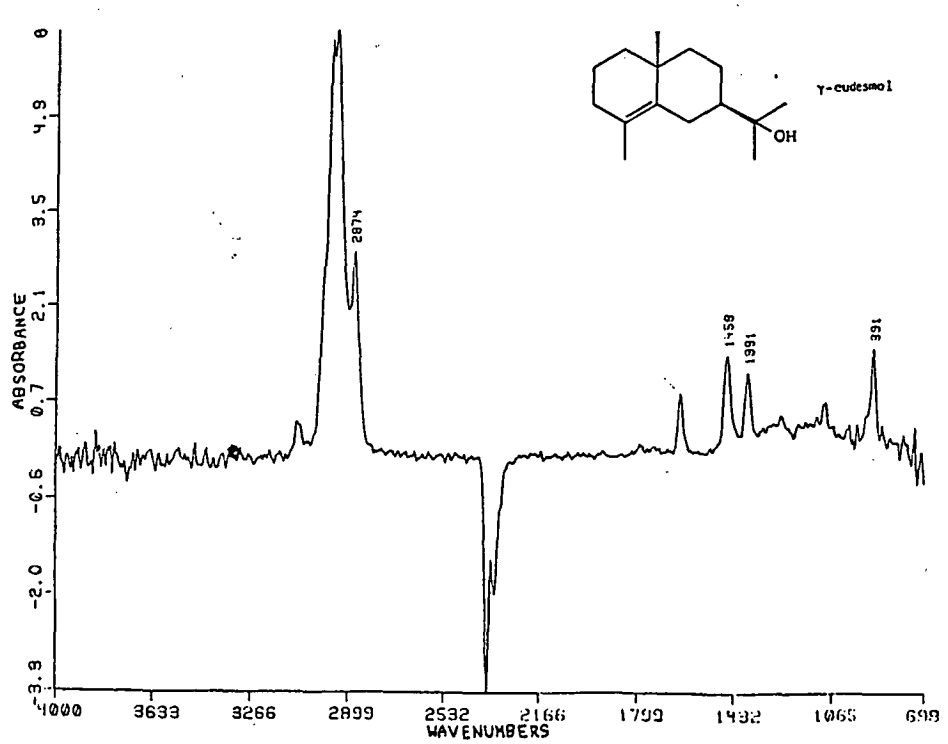


FIGURE IV.2.11

FTIR SPECTRUM OF γ-EUDESMOL



presence of absorptions at 3086 cm^{-1} and 895 cm^{-1}, which indicate an exocyclic double bond. The δ(O-H) and δ(C-O) of the alcohol were not observed. β-eudesmol spectra were observed from MW, GL, EP and PP oils.

γ-EUDESMOL

The Ftir spectrum of γ-eudesmol shown in Figure IV.2.11 was obtained from PP oil. The spectrum shows a similar absorption pattern as β-eudesmol, Figure IV.2.10. GL oil also produced a spectrum corresponding to this component.

CARYOPHYLLENE

An Ftir spectrum of caryophyllene was obtained from the EP and GL oils. The absorption bands and structure are shown in Figure IV.2.12. The spectrum is consistent with that found in the literature (Wenninger J.A. *et al.*, 1967).

BICYCLOGERMACRENE

The data presented in Figure IV.2.13 represent bicyclogermacrene. This component was identified from EP oil, but also occurs in BU and GL oils. This constituent was formerly thought to be germacrene B. The gc/ms evidence suggests bicyclogermacrene, and the Ftir confirms that the absorption is not due to γ-elemene, since there is an absence of exocyclic double bond absorption peaks. Neither is it due to germacrene B, since there is no trans double bond absorption at around 970 cm^{-1}.

GERMACRENE-D

Figure IV.2.14 shows the Ftir spectrum and structure of germacrene-D. The presence of the exocyclic double bond is confirmed by the absorptions at 3080 cm^{-1} and 891 cm^{-1}. The trans double bond in the ring system is revealed by the 970 cm^{-1} absorption. Note also the splitting of the δC=C at about 1600 cm^{-1} into two absorptions due to the conjugated double bonds. This determination is supported by gc/ms data.

FIGURE IV.2.12

FTIR SPECTRUM OF CARYOPHYLLENE

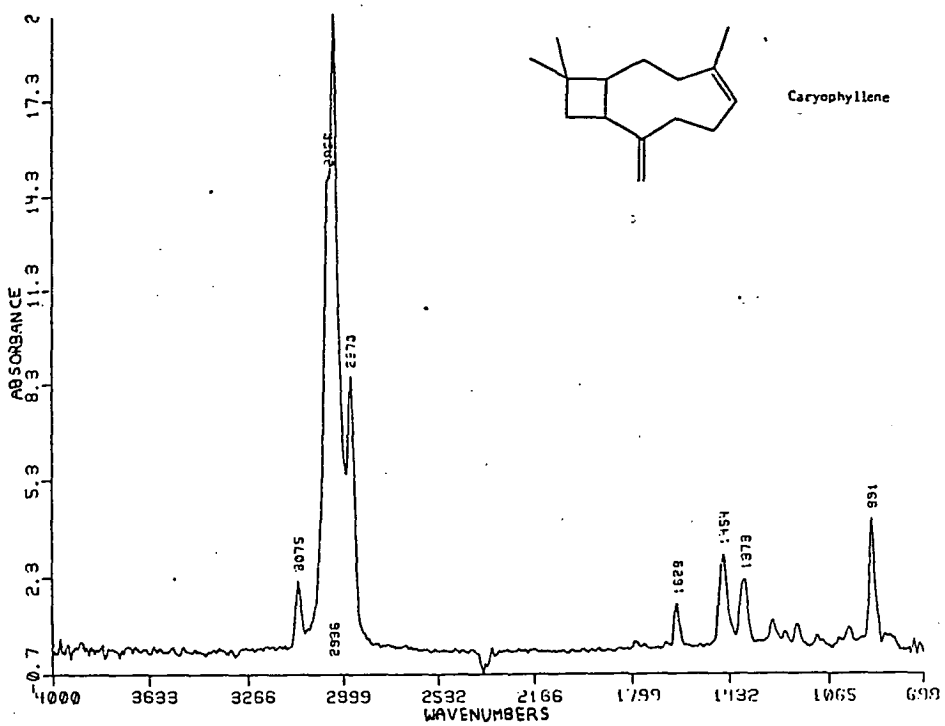


FIGURE IV.2.13

FTIR SPECTRUM OF BICYCLOGERMACRENE

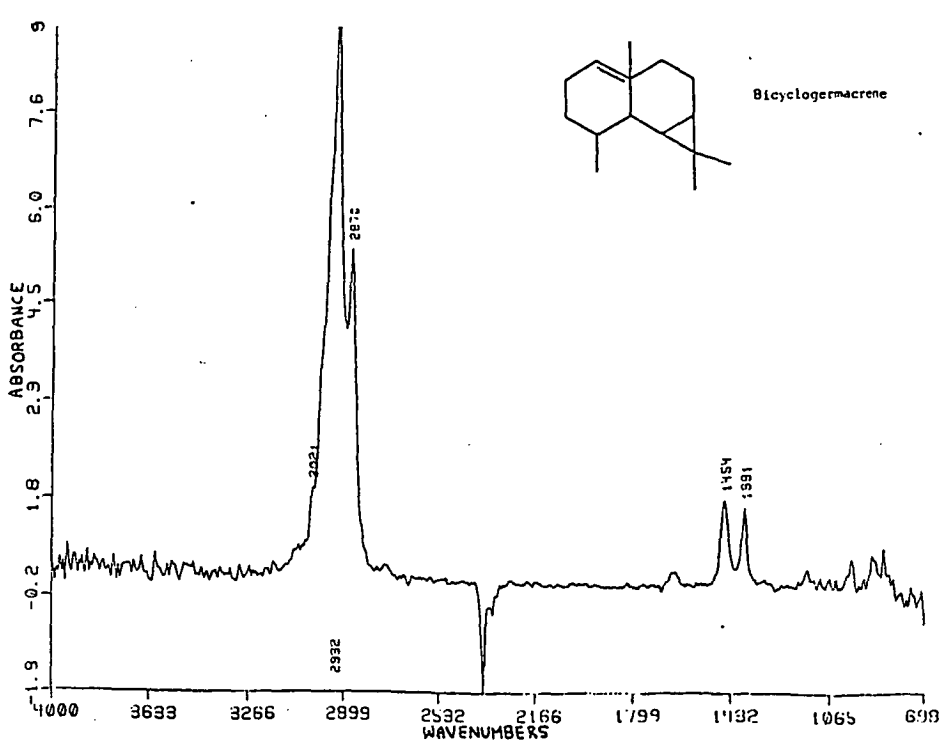
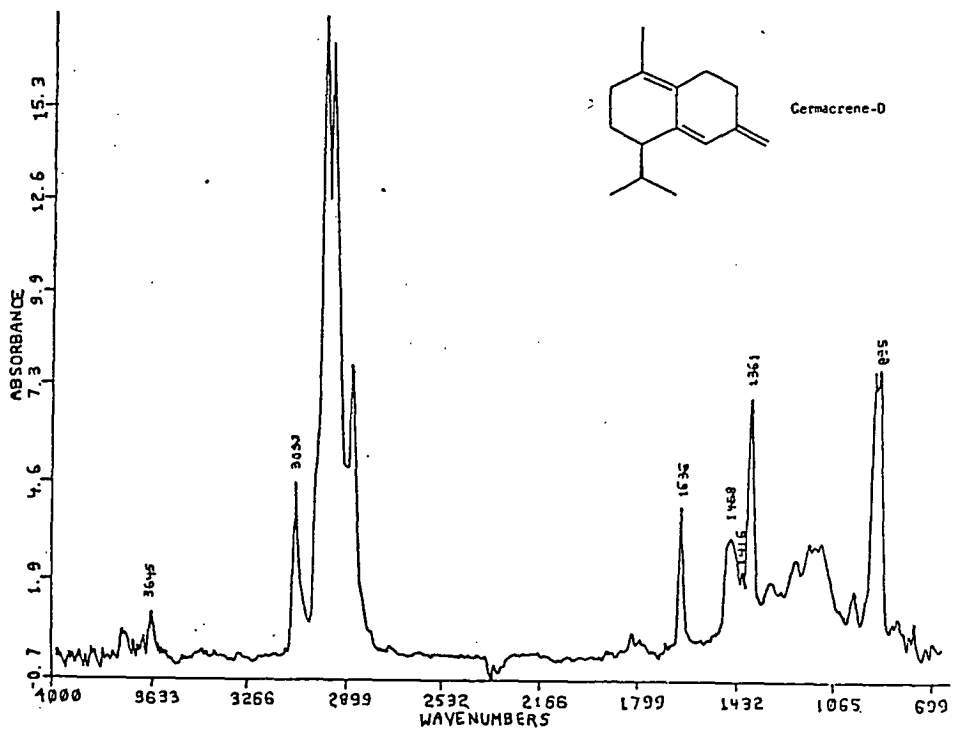


FIGURE IV.2.14

FTIR SPECTRUM OF GERMACRENE-D



II. UNIDENTIFIED COMPONENTS

During the course of the Ftir work, spectra were obtained for which definitive structures could not be assigned. The following observations were made:

In the MW oil, the peak at 31.52 min has been shown to be a sesquiterpene alcohol by gc/ms. The Ftir spectrum was too weak to show the presence of the δ(O-H) and δ(C-O) absorptions. However, the presence of an exocyclic double bond is suggested by the peaks at 3086 cm^{-1}, 1643 cm^{-1} and 891 cm^{-1}.

Also detected in the MW oil was a component (retention time 31.80 min), which exhibited an absorption band at 988 cm^{-1}, which reveals the presence of a trans double bond within the structure.

The first two of the six components from PP oil, for which Ftir spectra were obtained, did not have structures assigned. The first peak (retention time 13.53 min), was shown to be a compound which contains trans double bonds and does not appear to be an alcohol. Presumably it is an ether, and is likely to be 1,8-cineole by comparison with gc/ms, and gc/Ftir 1,8-cineole spectra.

The other peak (retention time 31.37 min) was shown to be a saturated hydrocarbon. The ms shows this to have a molecular formula $C_{15}H_{24}$, indicating a tetracyclic system.

The peak in EP oil with retention time 12.71 min, is an unknown $C_{15}H_{22}$ molecule, with exocyclic double bonds present. The absorption at 3010 cm^{-1} may be due to a cyclopropane ring system.

Analysis of the peak at 16.19 min from EP oil by gc/ms suggests the molecular formula of $C_{15}H_{24}O$. The Ftir spectrum shows a strong absorption at 1748 cm^{-1}, which indicates that a carbonyl functional group is present, though it is not an aldehydic one, since the characteristic aldehydic δ(C-H) absorptions are not observed. Presumably then, the component is a ketone. The position of the absorption suggests that it is a 5 or 6 membered ring ketone. In addition, the position implies that the carbonyl group is not conjugated to a double bond. (For instance, see shift in δ(C=O) position in isodehydrocarvone and piperitone, Mitzner B.M. and Mancini V., (1969)).

GL oil produced a major unidentified peak at 11.50 min, which gave rise to evidence that it is a sesquiterpene with an exocyclic

$C=CH_2$.

So far, no reference has been made to the composition of EN oil. This situation occurred because the spectra obtained from this oil sample were of insufficient intensity.

2.5 ANALYTICAL SCALE HPLC ANALYSES

The odour of the MW oil is characteristically different from the other five oils, in that it has a stronger spicey aroma. Hence, this oil was used for further fractionation work. Initially, however, all six oils were analysed by hplc to assess their relative compositions. The chromatograms of all six oils obtained with an acetonitrile gradient of 30 to 70% in 45 min are displayed in Figure IV.2.15.

Since the oils were steam distilled, they contain only monoterpene and sesquiterpene components which tend to separate out into two distinct groups with the acetonitrile/water solvent system used. Yet, considerable differences can be observed between the constituents present in the oils, as well as in their proportions. Lists of the retentions times and peak areas are given in Appendix C (a to f). Of particular interest is the group of four peaks present in the MW oil that occurs between 33 and 36 minutes, since these peaks do not appear in any of the other oils.

The approximate order of elution of various component types under these conditions, is indicated in Figure IV.2.15.

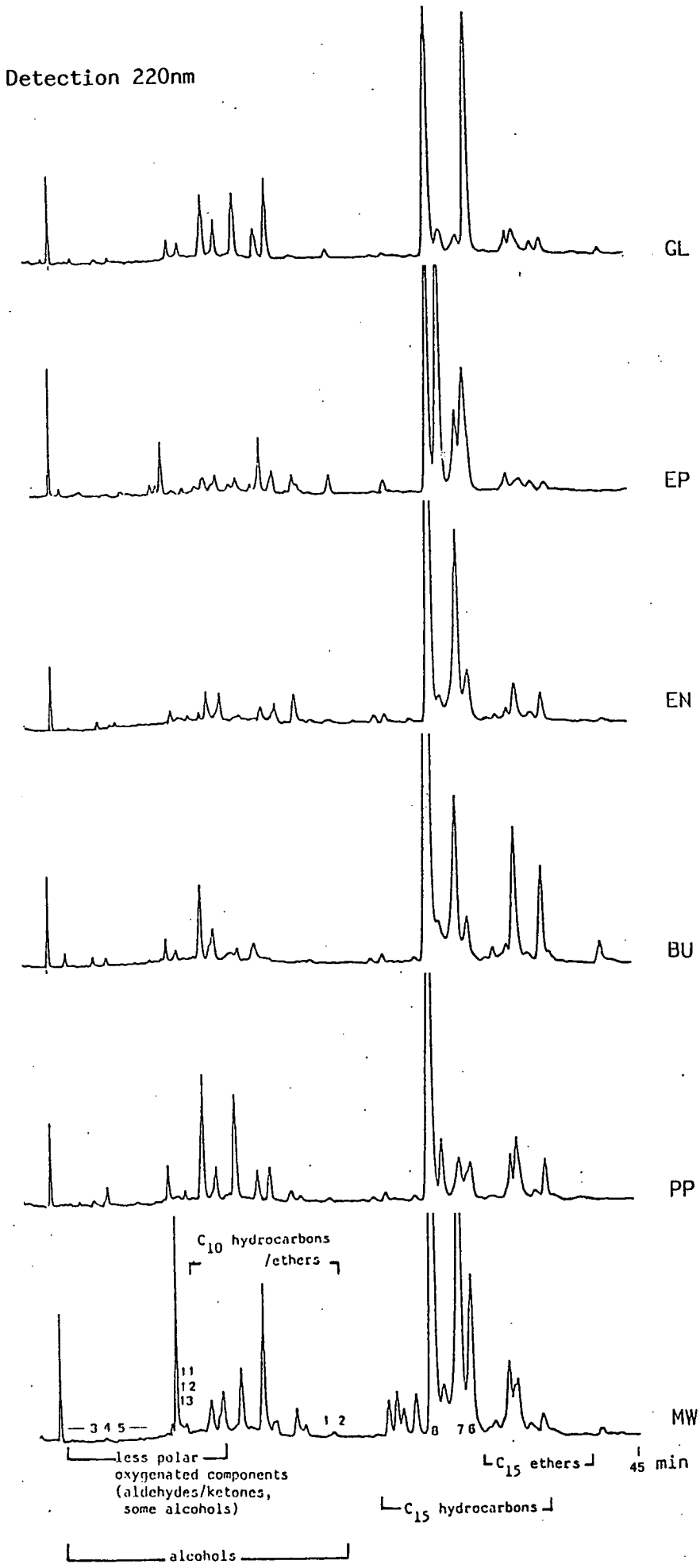


FIGURE IV.2.15

ANALYTICAL HPLC CHROMATOGRAMS OF OLEARIA CLONES

2.6 PREPARATIVE ISOLATION OF ESSENTIAL OIL COMPONENTS

2.6a PREPARATIVE SEPARATIONS OF WHOLE OIL USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Reversed phase high performance liquid chromatography was used to separate the components of MW oil. Strack D. *et al.* described a method employing acetonitrile/water as the mobile phase on a C_8 or C_{18} reversed phase column. The excellent resolution of a very complex terpenoid mixture using a linear gradient from 60% to 100% acetonitrile in less than one hour, prompted the use of a similar method. Acetonitrile is also transparent down to 190nm, and mixtures with water have the advantage of a negative enthalpy of mixing, which minimizes degassing problems.

The MW oil was initially separated into three fractions by the semi-preparative version of the method used for the analytical runs, with a Waters C_{18} µBondapak column (see Materials and Methods). The solvent gradient was modified to: 36% to 50% acetonitrile over 50 minutes and the injection volume increased to 250µl (21 mg). The samples were prepared in 2.00 ml tetrahydrofuran (THF).

The fractions obtained were extracted from the solvent/water mixture as follows. The sample was diluted with an equal volume of water, then, redistilled hexane was added (b.p. 69°C, 3 x 30 ml). The hexane extract was dried with anhydrous sodium sulphate and, after filtration, was evaporated at 35°C on the rotary evaporator until almost dry. The fractions were taken to dryness by passing nitrogen over them.

A typical chromatogram, showing the points at which the three fractions were collected, is given in Figure IV.2.16.

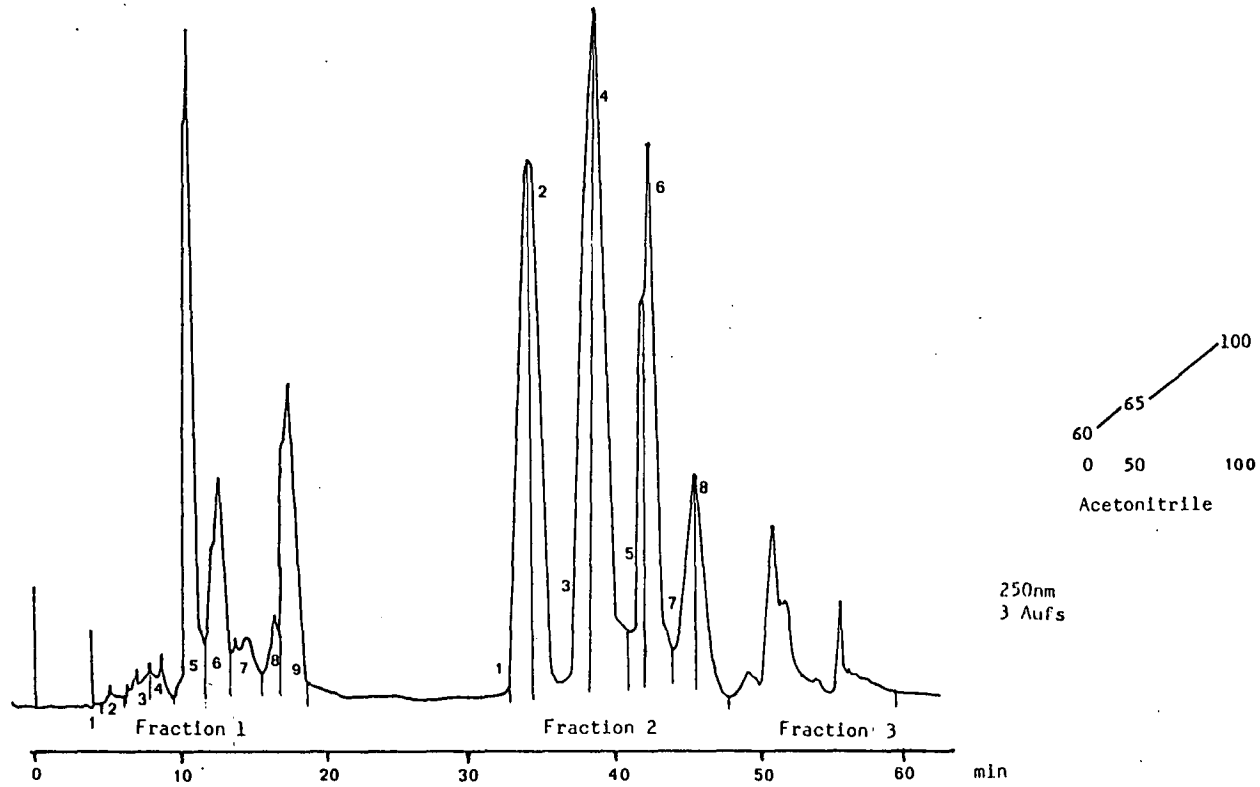
The fractions gave the following odour impressions:

Fraction 1 : Bushy, like Tea Tree, characteristic, exotic fruit, spicey
Fraction 2 : Heavy, sticky, sweet
Fraction 3 : Sweet, faint, woody, fresh, slight spice

From a total injection of 500 mg, 378 mg was recovered as :

FIGURE IV.2.16

TYPICAL HPLC TRACE SHOWING FRACTION COLLECTION POINTS



Fraction 1 159 mg

Fraction 2 199 mg

Fraction 3 20 mg

The components of fractions 1 and 2 were further separated by semi-preparative hplc to give nine and eight fractions respectively. The trace shown in Figure IV.2.16 also shows the collection points for these fractions. Even the best analysis techniques can be complemented by an olfactogram using the human nose as the most sensitive detector we have at our disposal. The odours of the fractions obtained from the hplc separation are presented in Table IV.2.6.

TABLE IV.2.6

ODOUR IMPRESSIONS OF HPLC FRACTIONS FROM MW OIL

Fraction 1

1	papery, mouldy
2	wine, port, brandy
3	sweet, fruity, apples, fresh, honey, slightly spicey
4	bush, strong citrus notes
5	sweeter than 4, pleasant, lemon
6	slightly camphoraceous, sweet, strong woody fruity
7	caramel, sweet, heavy, pleasant, floral
8	sweet, sugary with 'Olearia' notes, green banana
9	heavy, pungent, sweet

Fraction 2

1	vinyl
2	spicey, fruity, 'Olearia'
3	very strong biting, woody, tomato
4	meaty
5	woody, biting, meaty
6	similar to 7, but more earthy
7	earthy, wet wool
8	lighter than 9, but similar, menthol, tending towards native bush, spicey

Since there were peaks in Fraction 2 that were reminiscent of the typical odour of the MW oil, the hplc method was modified in order to optimise the separation of this area of the chromatogram. In addition, the major peaks in the oil occurred in this fraction, which facilitated the collection of sufficient quantities for nmr studies. The second and third peaks, in particular, were sought due to their spicey odour. The ethers are important aroma compounds which lay beneath the latter part of Fraction 2 and remained undetected.

2.6b ISOLATION OF TWO MAJOR OIL COMPONENTS

Fraction 2 (60 mg) was separated into eight fractions. These were recombined as 1+2, 3+4, 5+6 and 7+8, and relabelled 2.1, 2.2, 2.3 and 2.4, respectively. The semi-preparative C_{18} μBondapak column was used. Of the four fractions, 2.1 and 2.2 contained the elements with spicey aromas. The following weights were obtained for the four fractions:

2.1	15.6 mg	fruity
2.2	17.2 mg	tomato
2.3	7.3 mg	woody
2.4	3.4 mg	spicey, native bush

It was then intended that these samples would be further purified for analysis, however, 2.3 and 2.4 were complex mixtures which made separation, possibly unworthwhile. From preliminary nmr studies, fraction 2.3 was a mixture of unsaturated hydrocarbons and fraction 2.4 contained either hydrocarbons or ethers. Thus, only 2.1 and 2.2 were given further attention.

Fraction 2.1 was purified to homogeneity using an acetonitrile/water gradient: 40% to 65% in 30 minutes.

Since the resolution of peak 2.2 under these conditions was unsatisfactory, some solvent systems other than acetonitrile/water were investigated. The analytical column was used to run MW samples with methanol/water, tetrahydrofuran/water and iso-propanol/water.

Examples of the separation obtained can be seen in Figure IV.2.17.

In the first instance, the solvent system was 60% methanol/water, with the gradient running from 55% to 85% in 50 minutes. The resolution obtained for the whole oil was good. However, when Fraction 2.2 was run, the resolution was not considered good enough to use this system for the final cleanup.

The second solvent that was used was tetrahydrofuran. Resolution obtained here was even poorer than with methanol, and THF/water was abandoned.

The final solvent system was 60% propan-2-ol/water. Trial runs of the MW oil in this system gave encouraging results, so fraction 2.2 was purified using an 85% (solution A) isocratic run. It was observed that the peak resolved into one large peak and one smaller one. This contaminant peak did not elute until after the major peak had come off the column. This facilitated the collection process, since the cutoff collection point became easier to estimate than if the peak had eluted before the major peak. Typical chromatograms from the purification of the two peaks from fraction 2 are shown in Figure IV.2.18.

2.6c CHARACTERISATION OF COMPOUNDS FROM FRACTION 2

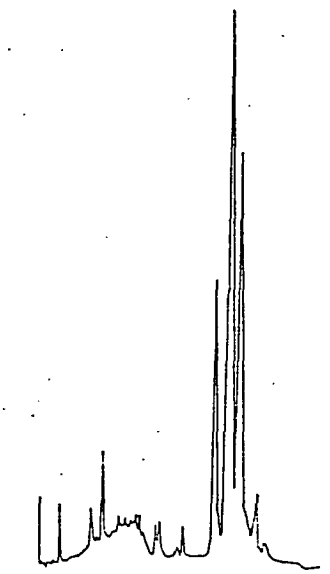
The broadband decoupled ^{13}C nmr spectrum of fraction 2.1 showed the presence of 15 carbon atoms and a DEPT edited spectrum confirmed the presence of 24 protons attached to carbon atoms (4 methyl, 4 methylene and 4 methine carbon atoms). This left three carbons, which had to be quaternary. See Figure IV.2.19.

A ^{1}H-^{13}C correlated 2D nmr spectrum yielded all of the direct proton-carbon connectivity information and most of the carbon-carbon connectivity was determined indirectly through an analysis of a ^{1}H-^{1}H correlated 2D nmr spectrum, as shown in Figure IV.2.20. This yielded a fairly complete partial structure and chemical shift arguments then led to the postulation of the bicyclogermacrene structure (Figure IV.2.21), for fraction 2.1. This was confirmed by comparison with ^{13}C nmr data published for bicyclogermacrene (Nishimura K. *et al.*, 1973). The chemical shifts are given in Table IV.2.7.

Mass spectral data was also in agreement with the proposed structure, with a molecular ion at m/e 204 in the mass spectrum, and

FIGURE IV.2.17

VARYING SEPARATION WITH VARIOUS SOLVENT SYSTEMS



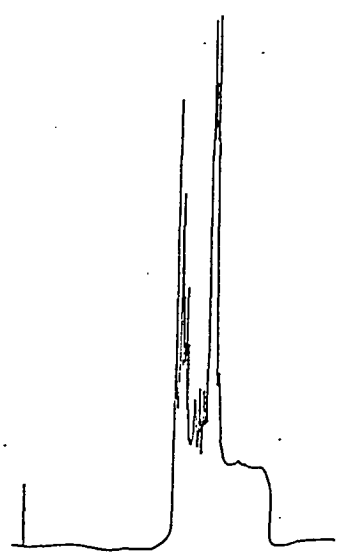
Isopropanol / Water

Solution A = 60% isopropanol

100

36

0 30 min

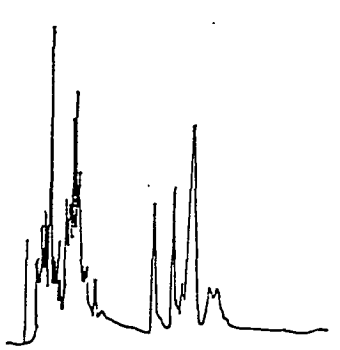


Tetrahydrofuran / Water

100

18

0 45



Methanol / Water

100

80

0 55

FIGURE IV.2.18

PURIFICATION OF TWO COMPONENTS FROM FRACTION 2

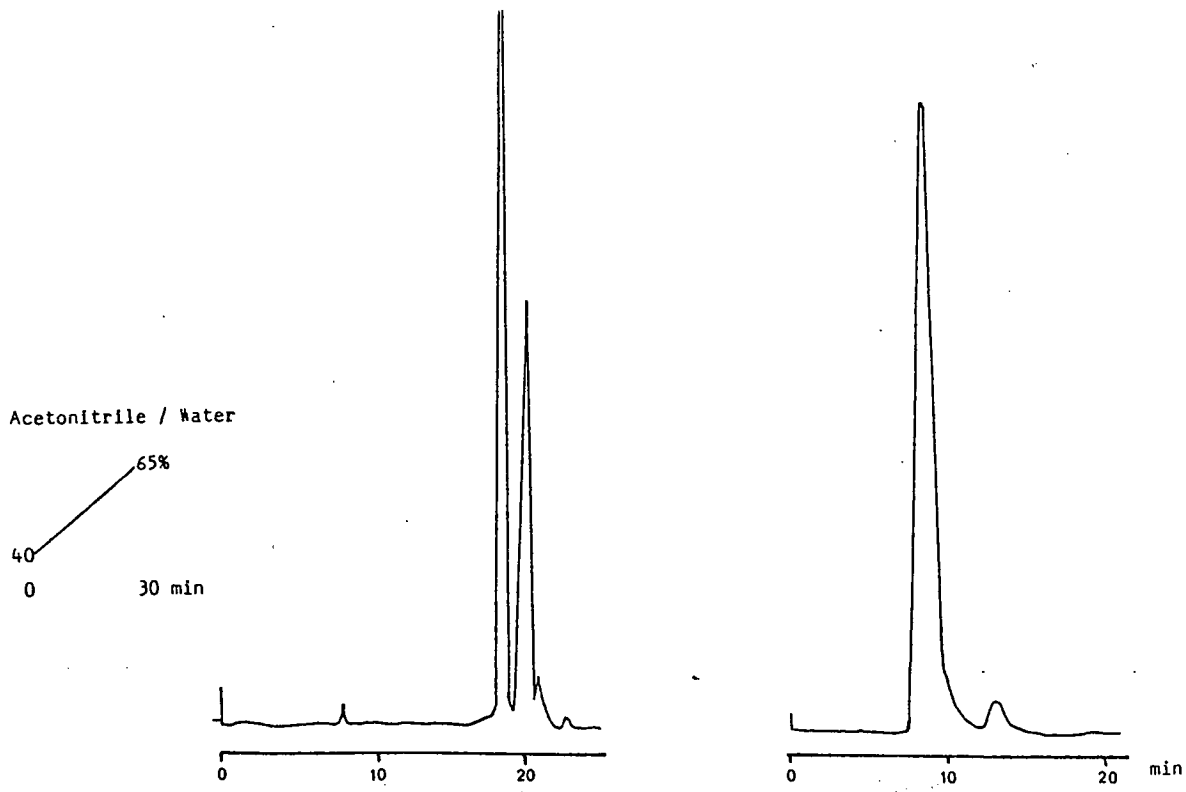


FIGURE IV.2.19

BROADBAND DECOUPLED ^{13}C NMR AND

DEPT EDITED SPECTRA OF BICYCLOGERMACRENE

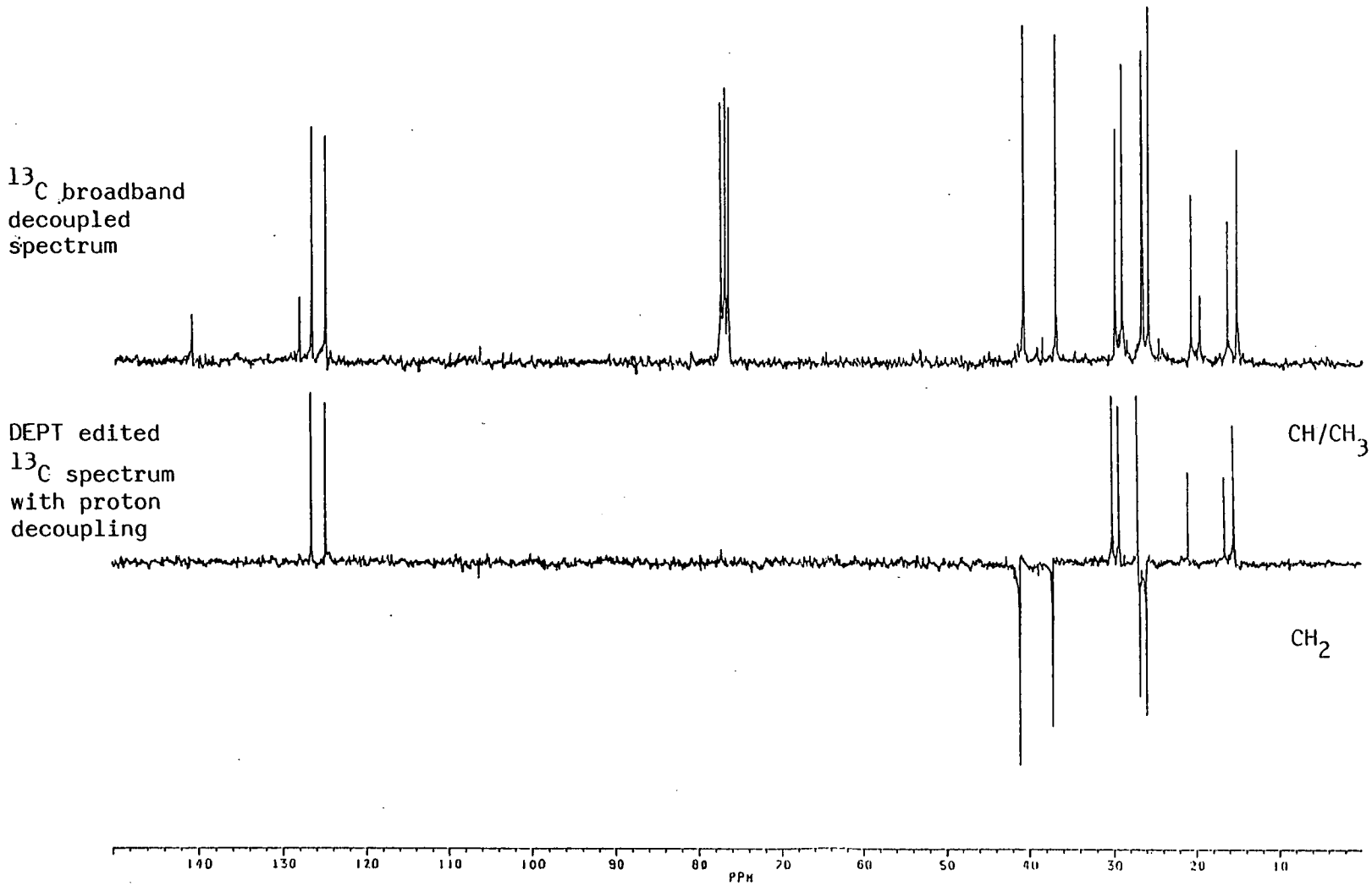
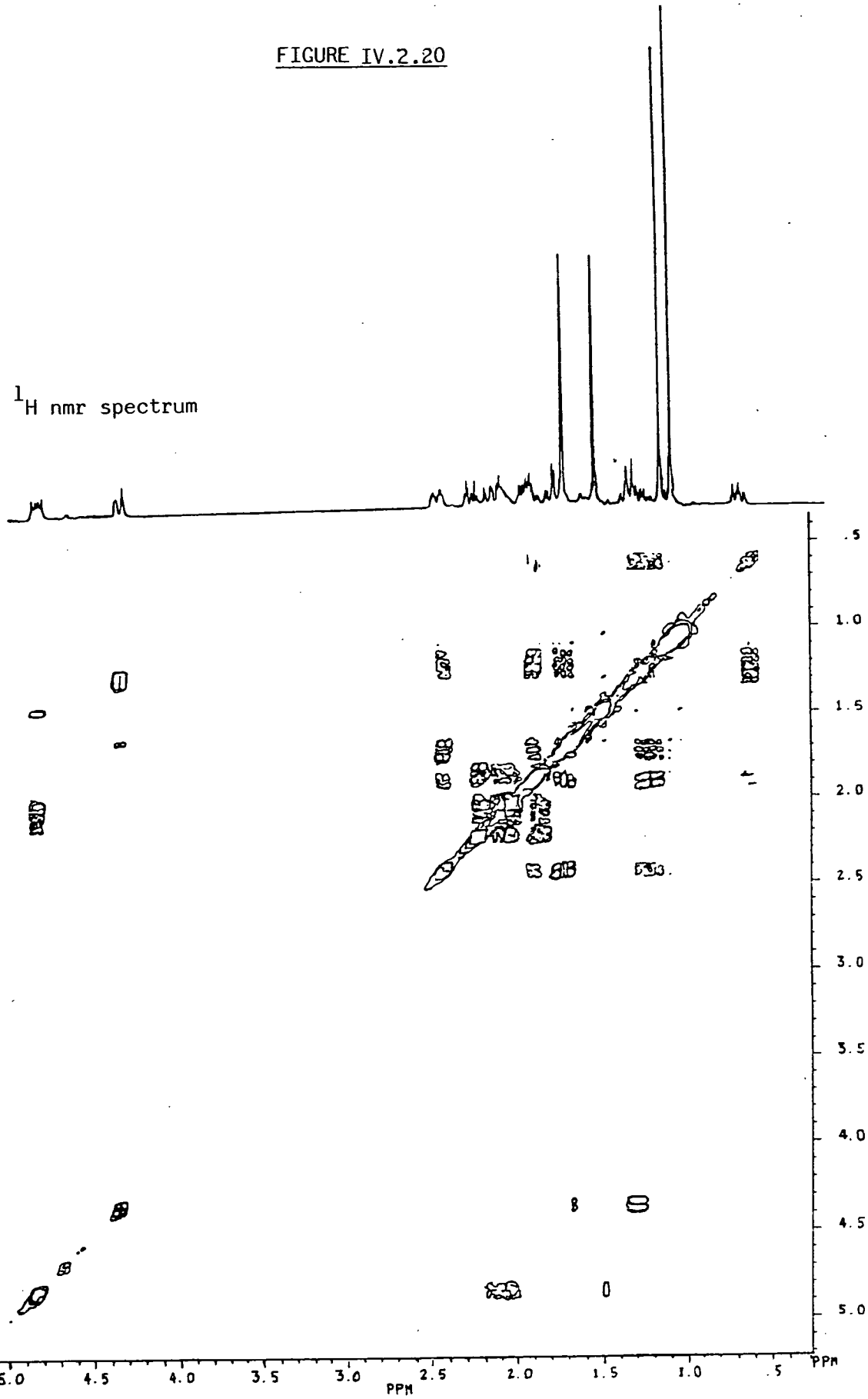


FIGURE IV.2.20



^{1}H-^{1}H COSY SPECTRUM OF BICYCLOGERMACRENE

a very similar fragmentation pattern to that published (Nishimura K., *et al.*, 1969).

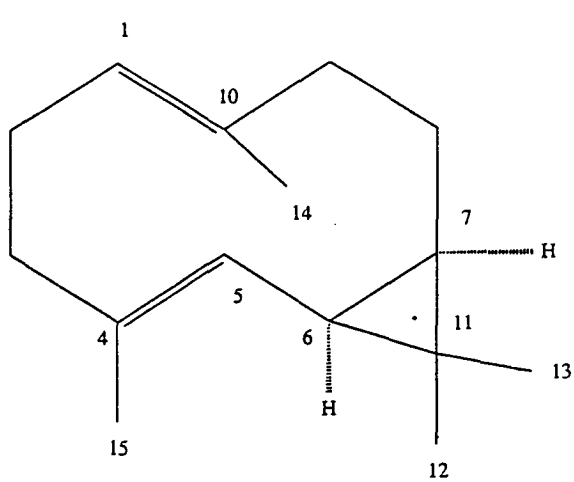
TABLE IV.2.7

CHEMICAL SHIFT DATA FOR BICYCLOGERMACRENE

	$\delta\ ^{13}C$	$\delta\ ^{1}H$
C_{10}	140.63	
C_4	127.95	
C_5	126.47	4.347
C_1	124.79	4.833
C_3	41.13	1.852, 2.223
C_9	37.21	1.717, 2.424
C_7	30.01	0.613
$C_{12/13}$	29.19	1.091
C_6	26.91	1.288
C_8	26.80	1.20, 1.91
C_2	25.98	1.98, 2.11
C_{15}	20.84	1.480
C_{11}	19.83	
C_{14}	16.54	1.663
$C_{12/13}$	15.41	1.026

FIGURE IV.2.21

BICYCLOGERMACRENE



The isomeric lepidozene having a *trans*-fused cyclopropane was ruled out because of the very different ^{1}H shifts of the cyclopropane methines (Matsuo A. *et al.*, 1984).

Bicyclogermacrene appears to occur in many Australian native species, (pers. comm. Davies N.), covering various families such as Myrtaceae, Asteraceae and Rutaceae. It has also been reported in commercial oil samples such as *Citrus junos*, (Nishimura K. *et al.*, 1969). The percentage concentration of bicyclogermacrene in these oils is low, whereas in Olearia oils, it occurs as a major constituent. This component was also detected and isolated from GL oil (see IV.2.7).

The major component of the fraction 2.2 was identified as germacrene-D by its characteristic set of olefinic proton resonances. These corresponded well with those reported in the literature (Yoshihara K. *et al.*, 1969, Bohlmann F. *et al.*, 1986).

Germacrene-D is widespread in essential oils of many different families. For instance, it occurs in citronella oil, *Cymbopogon nardus, C. winterianus* (Gramineae) and fennel.

The components detected (bicyclogermacrene and germacrene-D), did not have the desired spicey odour detected in Fraction 2. Therefore, some minor components, which had very little uv absorption (see IV.2.6e), must be responsible for this effect.

2.7 ISOLATION AND IDENTIFICATION OF BICYCLOGERMACRENE AND CARYOPHYLLENE FROM GL OIL

The major components of GL oil appeared to be bicyclogermacrene and caryophyllene, by mass spectroscopy. In order to confirm their presence, both in GL and the other five oils, a separation by hplc was undertaken.

The Brownlee Labs column was used with the conditions detailed in Materials and Methods. An acetonitrile/water gradient was used as shown in Figure IV.2.22. 101.4 mg of oil (in 2.5 mg hplc grade THF) was separated as shown. Two fractions were obtained, GL1 and GL2, which were analysed by nmr.

GL1	10.82 mg	(11%)
GL2	16.24 mg	(16%)

Fraction GL1 proved to be bicyclogermacrene by ^{1}H nmr, its identity being confirmed by comparison with the literature. (Nishimura K. *et al.*, 1973).

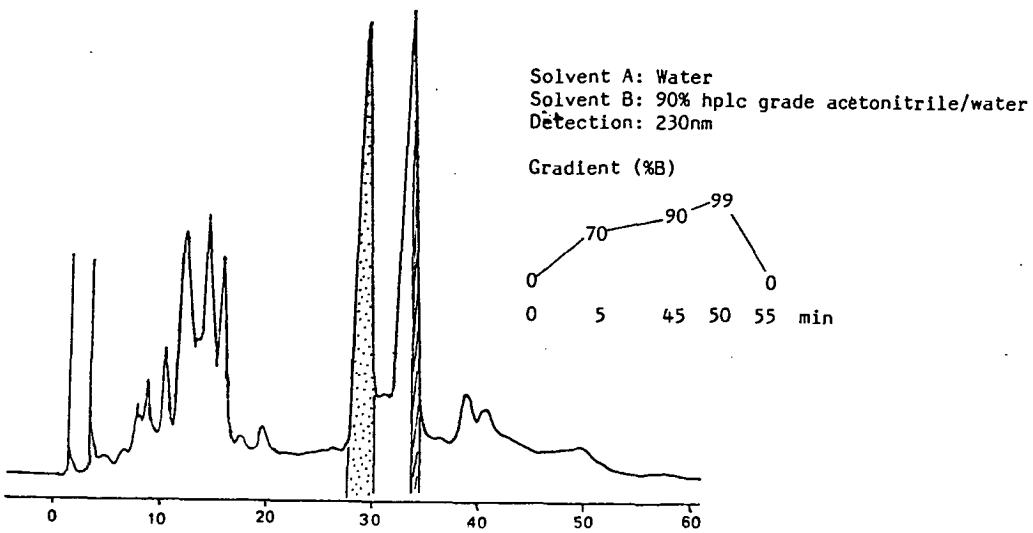


FIGURE IV.2.22

HPLC SEPARATION OF GL OIL

UV TRACE SHOWING COLLECTION POINTS

FOR FRACTIONS GL1 AND GL2

Fraction GL2 was shown to be caryophyllene by comparison of its ^{1}H nmr and ^{13}C nmr spectra with those in the literature (Formacek V. and Kubeczka K-H., 1982, Groweiss A. and Kashman Y., 1978).

The identification of caryophyllene in GL oil enabled the location and confirmation by gc of this component in the other oils. It occurs as a major component in GL oil, and to a lesser extent in MW, EN and BU oils. In EP and PP, caryophyllene is a minor peak.

2.8 PREPARATIVE HPLC FRACTIONATION OF FRACTION B FROM SILICA GEL PRE-FRACTIONATION

From the silica gel separation of the oils, it was evident that Fraction B was outstanding in that it contained the spicey components of the oil. Thus, methodology was developed to separate this fraction into its components by high performance liquid chromatography (hplc).

Reversed phase chromatography uses a non-polar column and a polar mobile phase. The organic solvent, when added as a gradient, decreases the polarity difference between the mobile and stationary phases during the run. Since the retention of a sample is related to this polarity difference, the effect of an increase in solvent concentration is to decrease the retention time of non-polar substances.

The observed separation on reversed phase columns can be the result of a number of factors. These include reversed phase interactions, adsorption on residual silanol groups and partition chromatography between the mobile phase and the adsorbed layers of solvent (Hancock W.S. and Sparrow J.T., 1984).

Fraction B derived from the silica gel pre-fractionation was run on an Amicon 22mm reversed phase C_{18} column. As an example, Figures IV.2.24 to IV.2.26 show three chromatograms obtained by running a methanol/water gradient, at 15 ml/min, for increasing amounts of Fraction B. The quantities injected were 20 mg, 100 mg and 300 mg. For comparison, 1 mg was run on a Radpak column, at 2 ml/min, as shown in Figure IV.2.23. From the figures, the resolution obtained with 20 mg on the Amicon column is similar to that of 1 mg on the Radpak column.

The loss of resolution during the 300 mg run was countered by the increased throughput possible. The advantage here lies in a

FIGURE IV.2.23

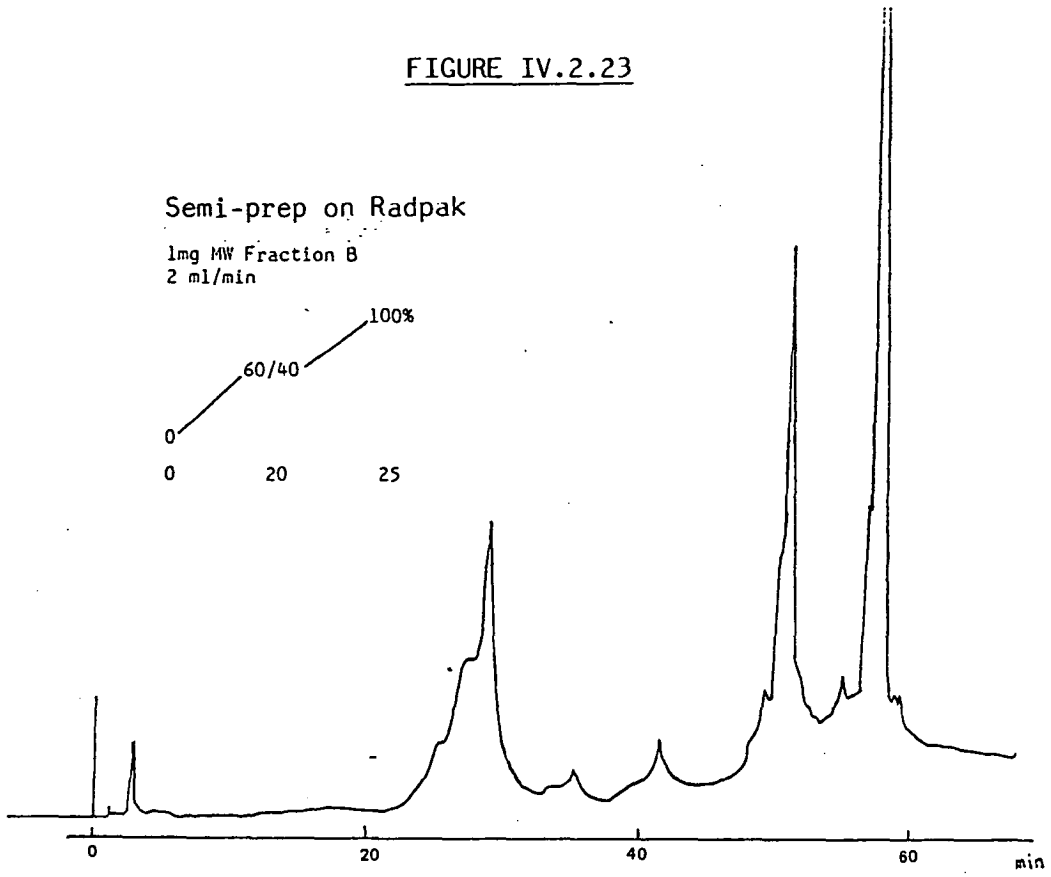


FIGURE IV.2.24

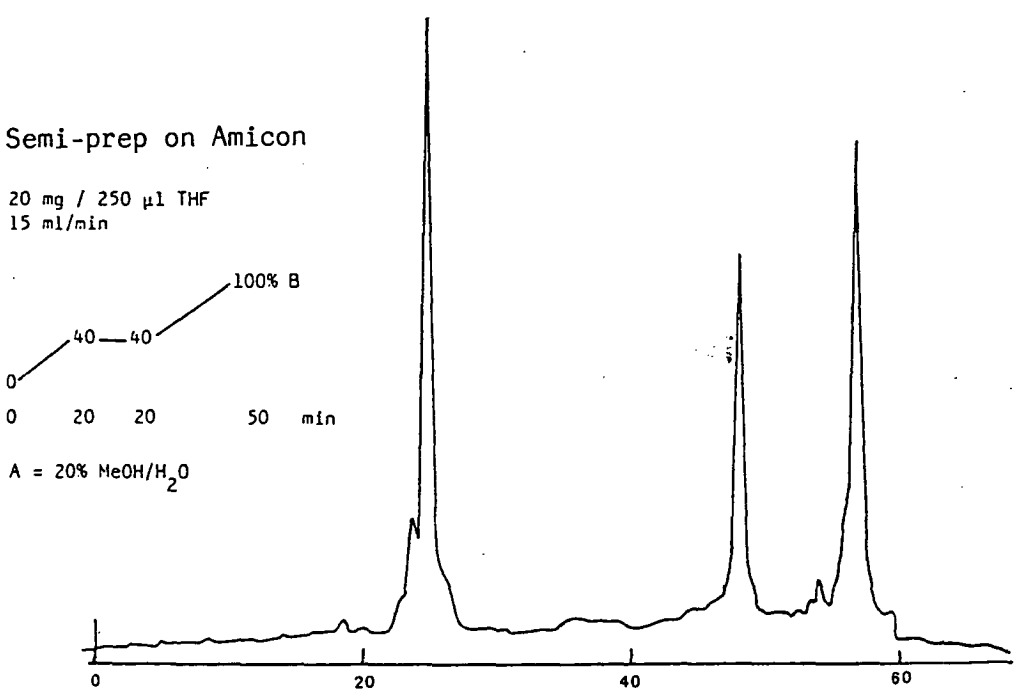
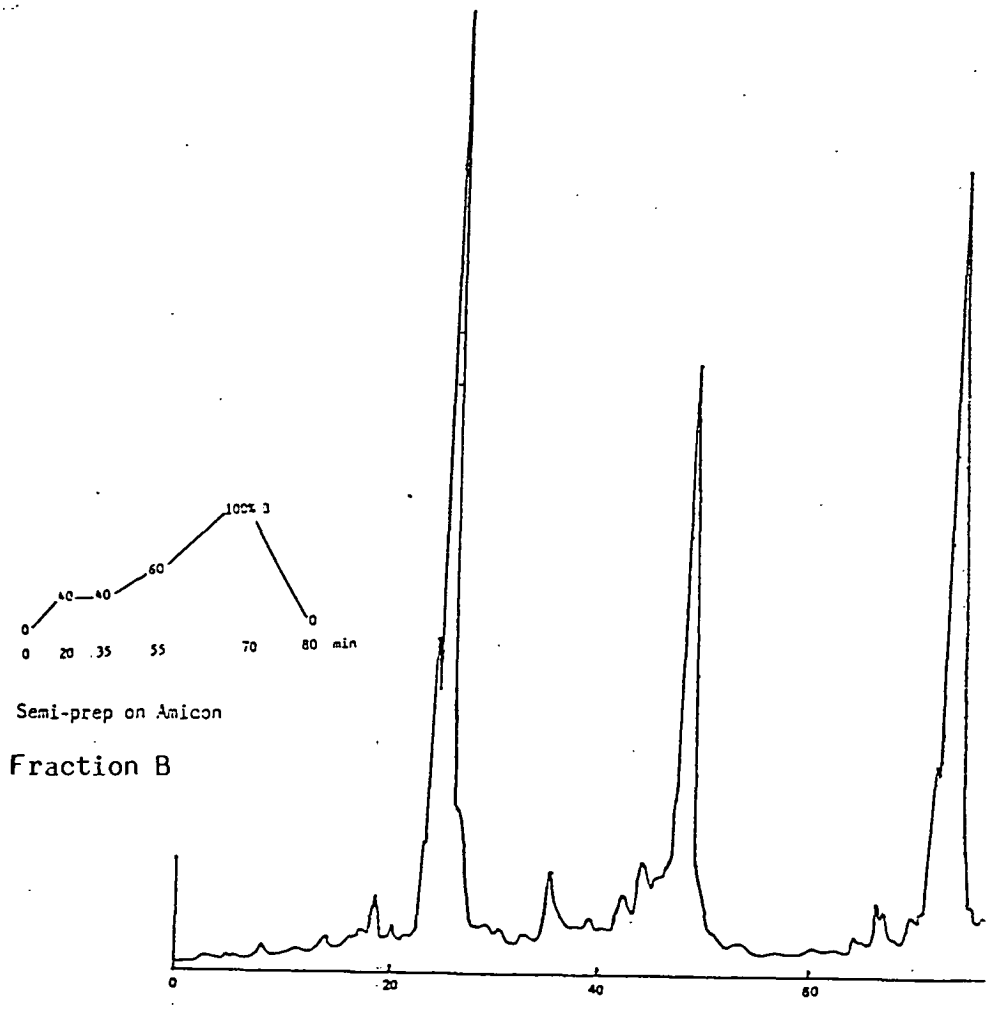


FIGURE IV.2.25



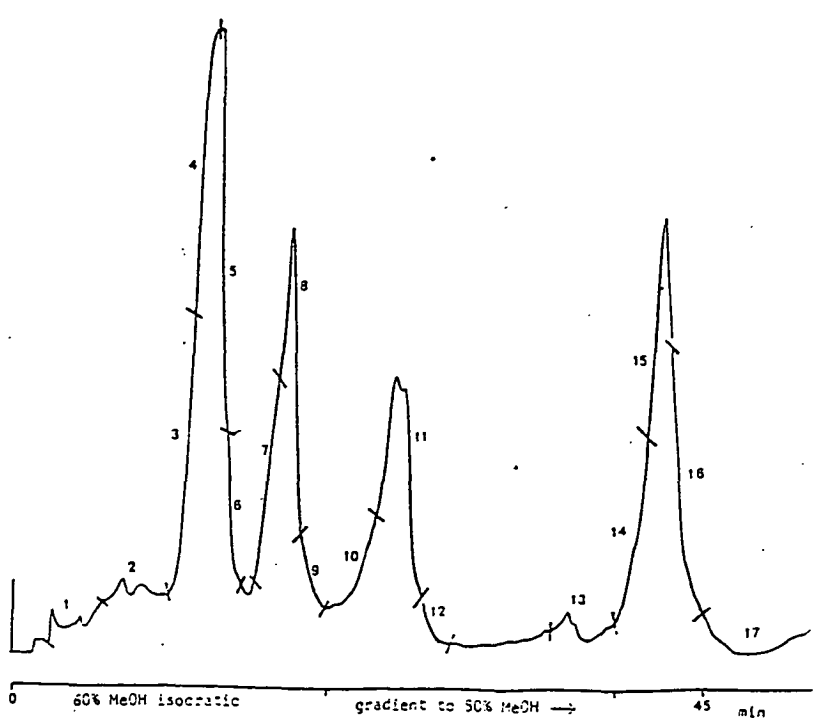


FIGURE IV.2.26

reduction of total processing time for fraction collection. The fractions were not expected to be pure components; rather they were bands containing several components.

Fraction B was separated into seventeen fractions, initially. Some of these were subsequently combined.

Figure IV.2.26 shows the trace of the preparative (300 mg) run. The collection points for the fractions are indicated. The sample concentration was 0.500 g of MW Fraction B in 2.0 ml THF.

The column operated most effectively when the solvent flow rate was 15 ml/min. In order to obtain such a flow, the pump system incorporated three pump units, each with a maximum pump speed of 10 ml/min, in the following way:

Pump 1 - MeOH; 7.5 ml/min

Pump 2 - MeOH

Pump 3 - 20% MeOH/Water; 7.5 ml/min from pumps 2 plus 3.

Detector - 250 nm; 2 AUFS

The gradient used is shown in Figure IV.2.26.

The weights of the fractions recovered from a total of 300 mg injected (in mg) are shown below:

1.	3.9	9.	5.4
2.	4.7	10.	22.0
3.	4.9	11.	30.1
4.	9.0	12.	85.3
5.	6.2	13.	3.4
6.	1.3	14.	7.8
7.	2.6	15.	24.6
8.	3.4	16.	14.4
		17.	8.2

Some of the seventeen fractions were then combined, and some were discarded, to leave the samples 3-5, 7-8, 9-10 and 11-12. These were re-labelled I, II, III and IV.

The combined samples taken from Fraction B were further separated to homogeneity on a Radpak normal phases silica column. Various solvent systems were tried, and a suitable one found for each separation. The solvents were hexane/tetrahydrofuran, hexane/diethyl ether and hexane/saturated acetonitrile in hexane (approximately 5%).

2.9 ANALYTICAL NORMAL PHASE SEPARATIONS

Fraction B contained the oxygenated components of the oil, thus, a Radpak 5 silica gel column was used instead of the reversed phase one. This was run at the optimum rate of 2 ml/min.

2.9a SEPARATION OF FRACTION I

This fraction was chromatographed using 2% ether/hexane. This solvent system gave a good baseline, and less residue than THF on the column due to contaminants. The ether was simply redistilled from iron (II) sulphate (hydrate) before use.

The actual weight of material present in this fraction was 4.7 mg. This was dissolved in 1 ml hexane and injected onto the column in 25µl aliquots.

A list of fractions recovered, their weights and odour impressions is presented below:

FRACTION	a	0.6 mg	lemon/eucalypt
	b	0.4 mg	floral
	c	0	astringent/disinfectant
	d	1.3 mg	floral (cheap perfume)

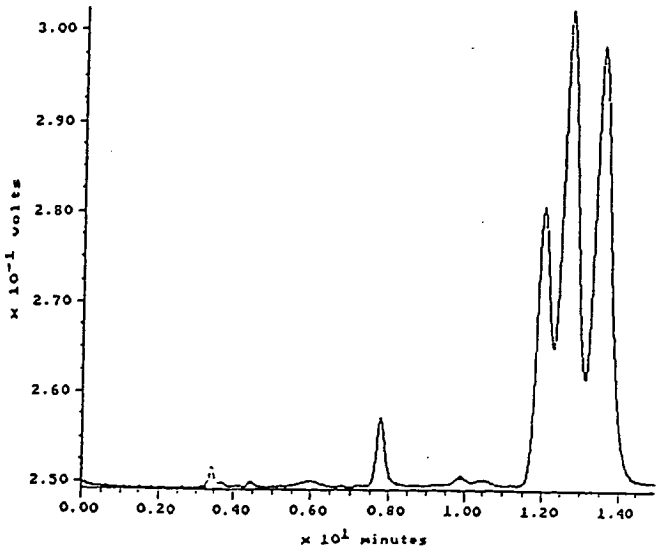
Figure IV.2.27 shows a series of three chromatograms. The first is the analytical trace, the second is a typical preparative run, and the last is an RI trace of the preparative run. The fraction collection points are indicated on the RI trace, and designated 1 to 4. The quantities collected precluded further work on these fractions.

FIGURE IV.2.27

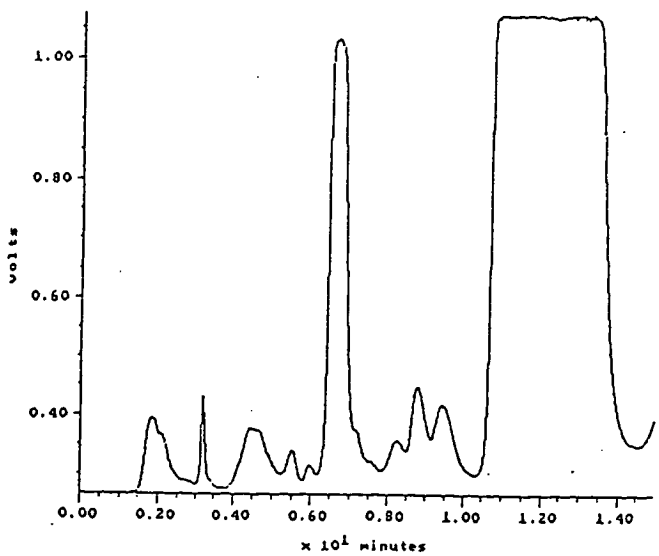
HPLC SEPARATION OF FRACTION I

Analytical trace

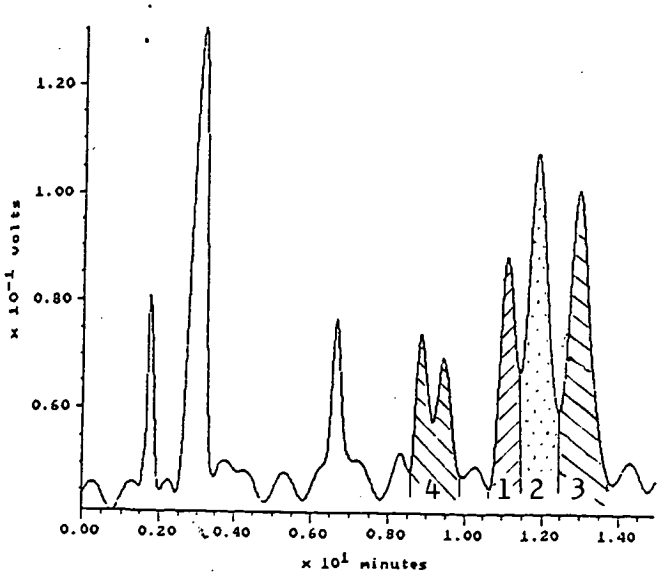
UV220nm



5mg Prep.



RI



2% Ether / Hexane

Analytical, Preparative and Refractive Index traces.

2.9b SEPARATION OF FRACTION II

Approximately 6.0 mg of fraction 7-8 was separated by hplc, using 5% THF/hexane. The run was isocratic, and about 2.0 mg was injected in 250µl hexane each time. A typical trace is shown in Figure IV.2.28. A list of fractions recovered, their weights and odour impressions is given below:

FRACTION	a	0.5 mg	floral/perfumed
	b	0.6 mg	spicey
	c	0.1 mg	spicey (but floral)

Fraction b was very interesting in its odour impression and some preliminary ^{1}H nmr, ir and ms data indicated that it might be a prenylated tropolone derivative. Further studies would have required separation of considerably more material.

2.9c SEPARATION OF FRACTION III

Some 27 mg of material was chromatographed in five parts. Initially, 5% ether/hexane was used as the solvent system, however, unsatisfactory resolution was obtained. This is shown in Figure IV.2.29, which presents the UV220 (UV detection at 220nm) and RI (refractive index) traces. Two fractions were collected from about half the inital starting material.

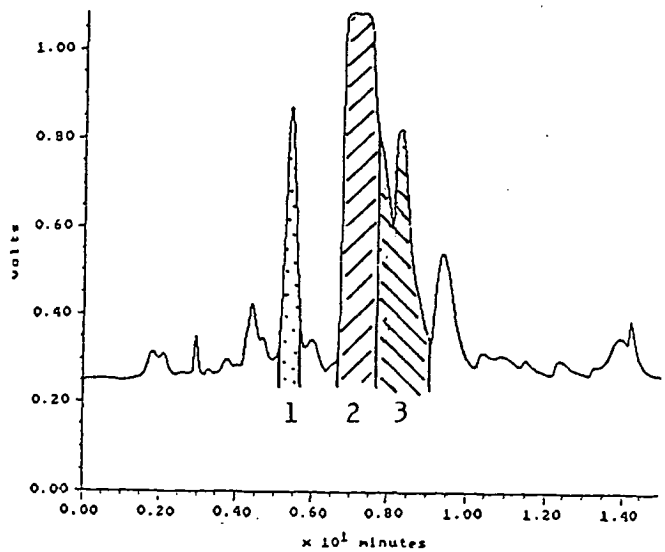
FRACTION	a	7.0 mg
	b	1.0 mg

The remainder of the sample was processed with a mixture of hexane/acetonitrile, which gave better results. Two fractions were obtained with a 50% saturated hexane/CH_3CN system. Figure IV.2.30 shows the UV and RI traces obtained.

FRACTION	a	5.5 mg
	b	3.6 mg

Fraction III was shown to be primarily two components in the

UV220nm
6mg



5% THF / Hexane

FIGURE IV.2.28
HPLC SEPARATION OF FRACTION II

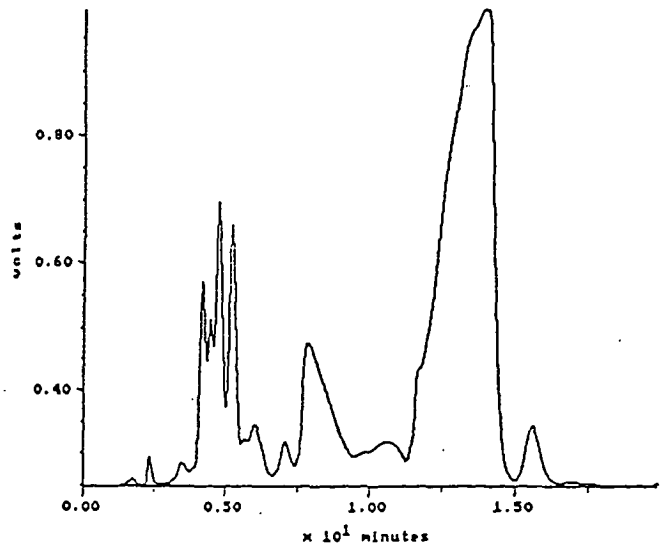
Collection points are shown

ratio 2:1 by gc/ms and by ^{1}H nmr analysis. The major component (Fraction a) was identified as caryophyllene oxide (mass spectrum given in Figure IV.2.39), by comparison of its ^{13}C nmr spectrum with that published in the literature (Bohlmann F. and Zdero C., 1978a). The results agreed closely except for a typographical error in which they transposed the ^{13}C shift data for C_4 and C_5.

The 300MHz ^{1}H nmr spectrum was similar to the 60MHz spectrum published (Suga T. *et al.*, 1975), and is shown in Figure IV.2.31. In the course of determining its structure, unambiguous assignment of both ^{1}H and ^{13}C spectra were established in a manner analagous to that described for bicyclogermacrene. This is summarised in Table IV.2.8 and Figure IV.2.32.

The other component of fraction III was identified as spathulenol by comparison of its mass spectral and ^{1}H nmr data with literature reports (LeQuere J.L. and Latrasse A., 1986). Figure IV.2.33 shows the proton nmr spectrum of the mixture of caryophyllene oxide and spathulenol. The characteristic peaks that arise due to the presence of spathulenol are indicated, and are in agreement with literature values. Spathulenol has been isolated from other members of the Asteraceae, namely *Artemisia vulgaris* L. and *A. dracunculus* L. (Juell S.M.K. *et al.*, 1976).

UV220nm



50% saturated Hexane / CH_3CN

RI

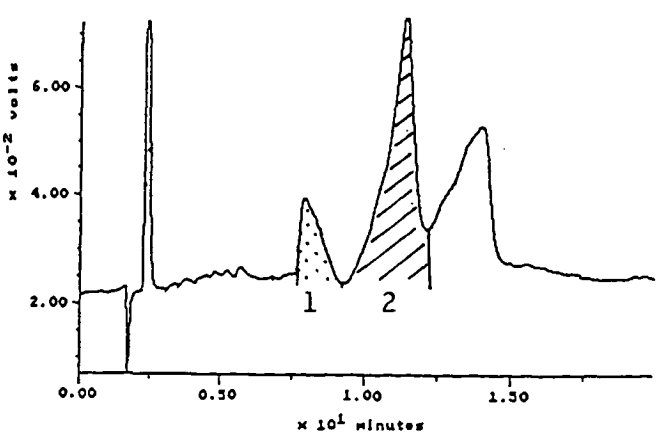
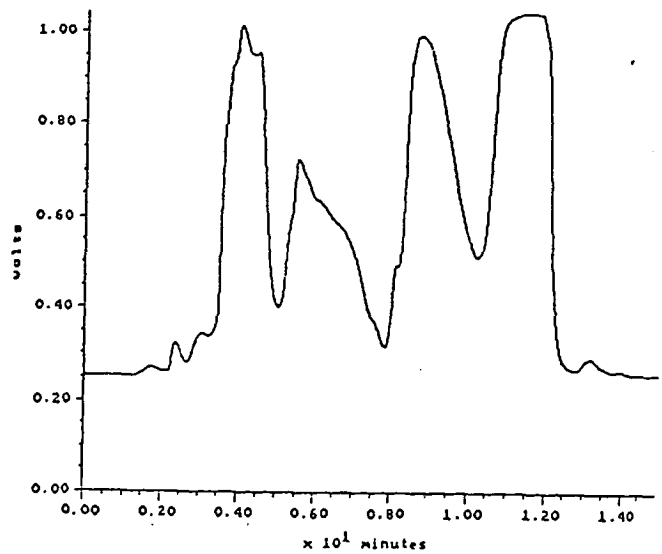


FIGURE IV.2.29

HPLC SEPARATION OF FRACTION III

UV and RI trace showing fraction collection points

UV220nm



5% Ether / Hexane

RI

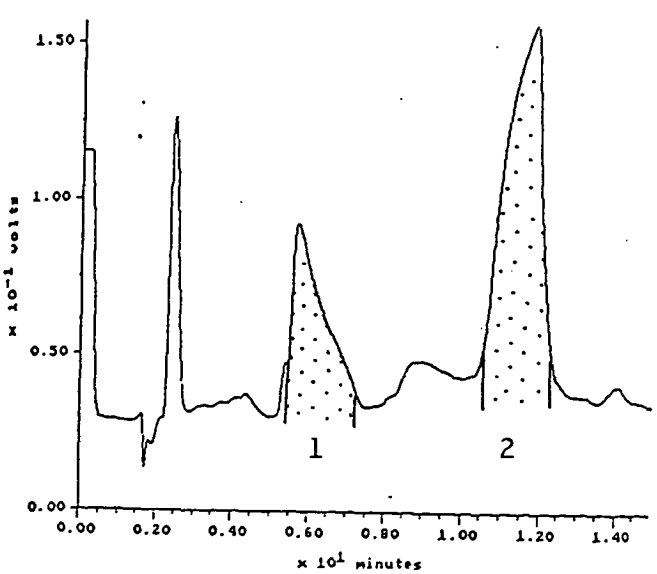


FIGURE IV.2.30

HPLC SEPARATION OF FRACTION III

UV and RI trace showing fraction collection points

FIGURE IV.2.31

^{1}H NMR SPECTRUM OF CARYOPHYLLENE OXIDE

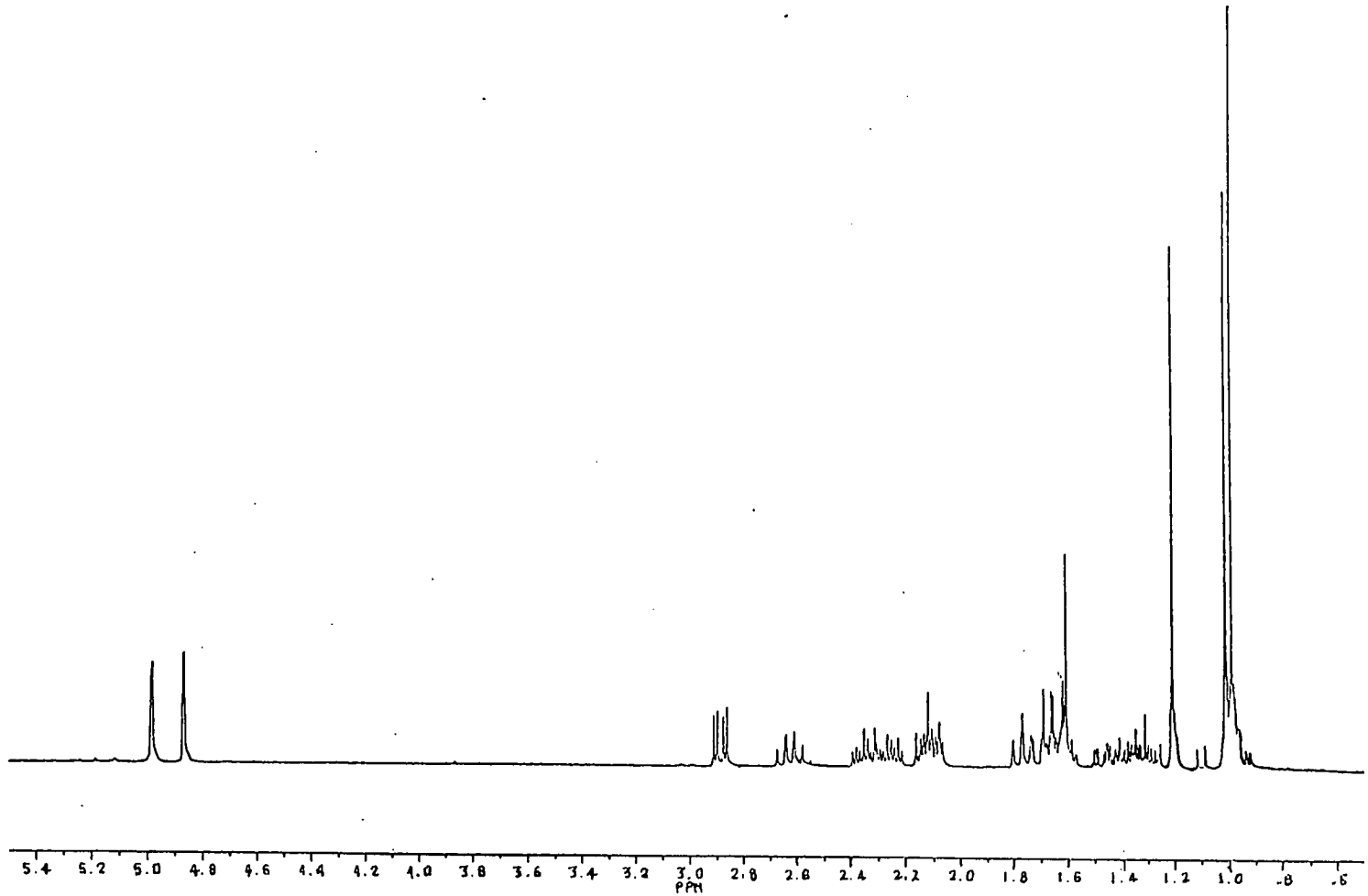


TABLE IV.2.8

^{1}H AND ^{13}C ASSIGNMENTS FOR CARYOPHYLLENE OXIDE

	$\delta^{13}C$	$\delta^{1}H$
C_8	151.85	
C_{12}	112.75	4.860, 4.976
C_5	63.77	2.880
C_4	59.85	
C_1	50.78	1.765
C_9	48.74	2.587
C_{10}	39.77	1.62
C_3	39.16	2.09, 0.96
C_{11}	34.02	
C_6	30.20	2.22, 1.31
$C_{13/14}$	29.79	0.987
C_7	29.66	2.32, 2.14
C_2	27.22	1.62, 1.42
$C_{13/14}$	21.63	1.009
C_{15}	17.00	1.205

FIGURE IV.2.32

CARYOPHYLLENE OXIDE

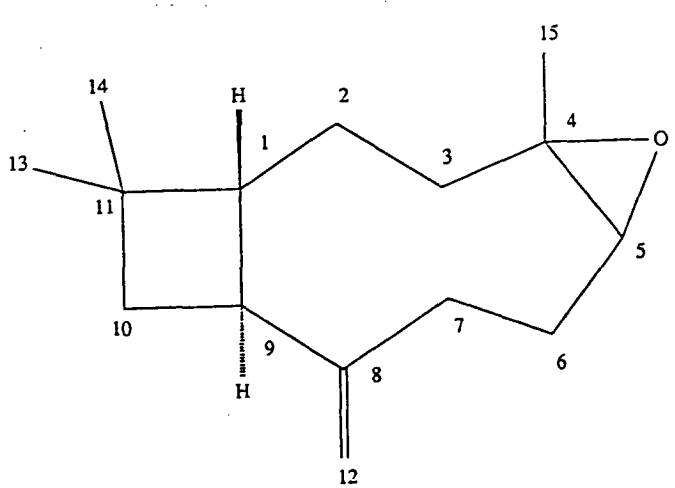
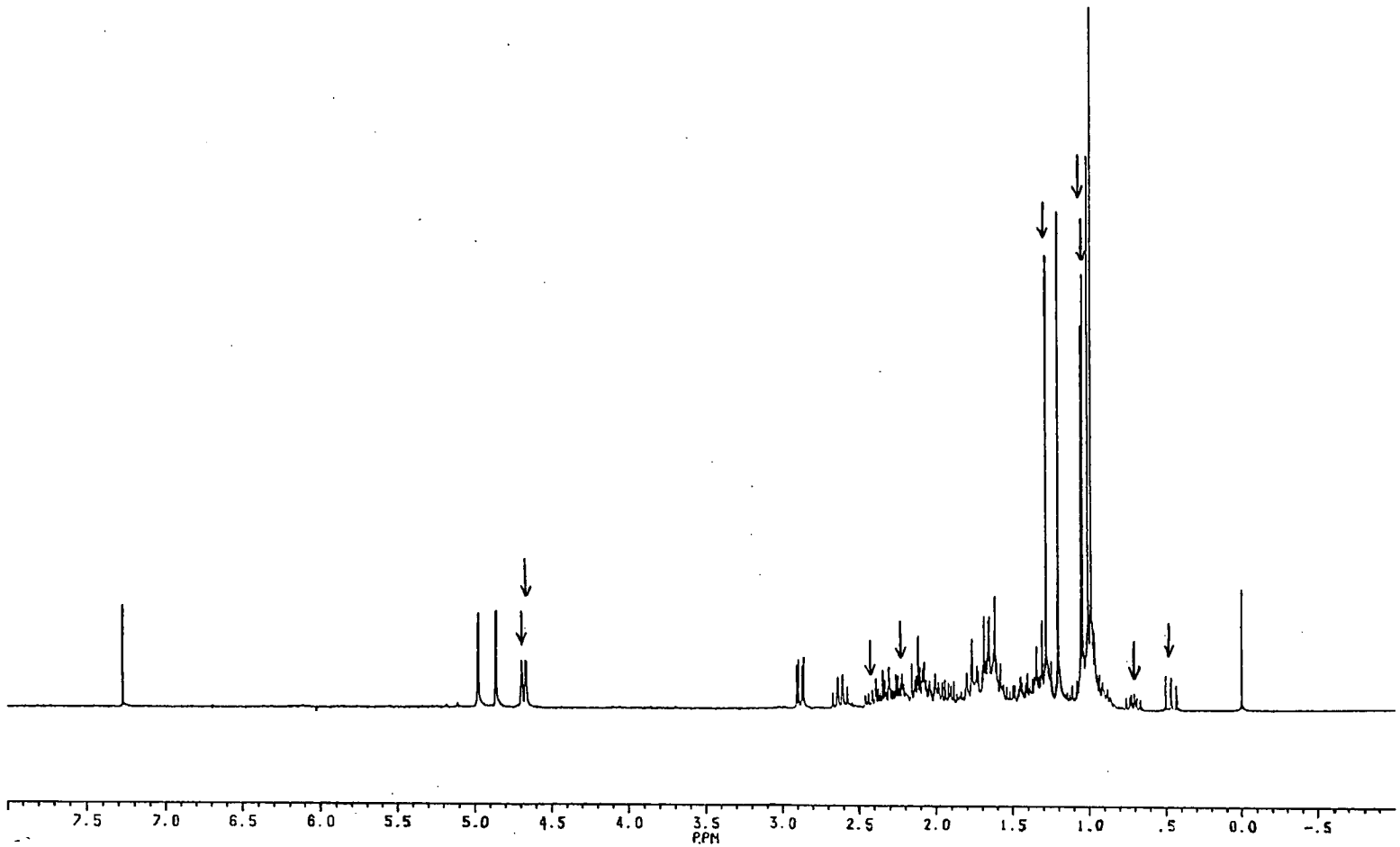


FIGURE IV.2.38

^{1}H SPECTRUM OF FRACTION b

CONTAINING CARYOPHYLLENE OXIDE AND SPATHULENOL

Note: peaks characteristic of spathulenol are indicated.



2.9d SEPARATION OF FRACTION IV

Fraction IV consisted essentially of two components as indicated by ^{1}H nmr and gc analyses. A total of 50.6 mg of material was dissolved in 2 ml hexane. Aliquots of 200µl were run, which delivered about 5 mg of material onto the column at each injection. The mobile phase was 20% saturated hexane/CH_3CN. Since one component showed virtually no UV absorbance above 220 nm, RI detection was used. Typical analytical and preparative traces are shown in Figure IV.2.34. Three fractions were collected:

FRACTION	a	12.2 mg	fruity
	b	19.6 mg	spicey
	c	6.1 mg	spicey

The residue of other peaks was 5.5 mg

Fractions a and c were shown to be pure by ^{1}H nmr, and fraction b consisted of a and c in the ratio 1:6. Both compounds had molecular ions at m/e 222, but had very different fragmentation patterns.

The two components were shown to have the same molecular formulae $C_{15}H_{26}O$, and similar ^{1}H and ^{13}C nmr data. A combination of standard ^{1}H and ^{13}C nmr techniques allowed a partial carbon skeleton to be determined for each.

Fraction a was then confirmed to be the oxygenated guiane sesquiterpene kessane, by comparison of its ^{13}C nmr spectrum and mass spectrum with published data (Zalkow L.H. *et al.*, 1980, Hikino H. *et al.*, 1963). The mass spectrum of kessane is shown in Figure IV.2.39.

Some discrepancy with the reported ^{1}H nmr spectrum (Hinino H. *et al.*, 1963), was noted. Resonance peaks were reported for kessane at 0.77 (d), 1.02 (s) and 1.18 (s) ppm, while those of fraction a occurred at 0.79 (d), 1.12 (s) and 1.25 (s, 2 x CH_3) ppm. (The proton nmr spectrum for kessane is presented in Figure IV.2.35). In the literature, however, the solvent and conditions are not reported, precluding a critical comparison. Comparison with the ^{13}C nmr, however, enabled an unambiguous identification. The nmr data obtained for kessane is summarised in Table IV.2.9.

An isomeric form of kessane, 5-epikessane has been reported by ApSimon J.W. *et al.*, 1977. However, the proton nmr spectrum of this

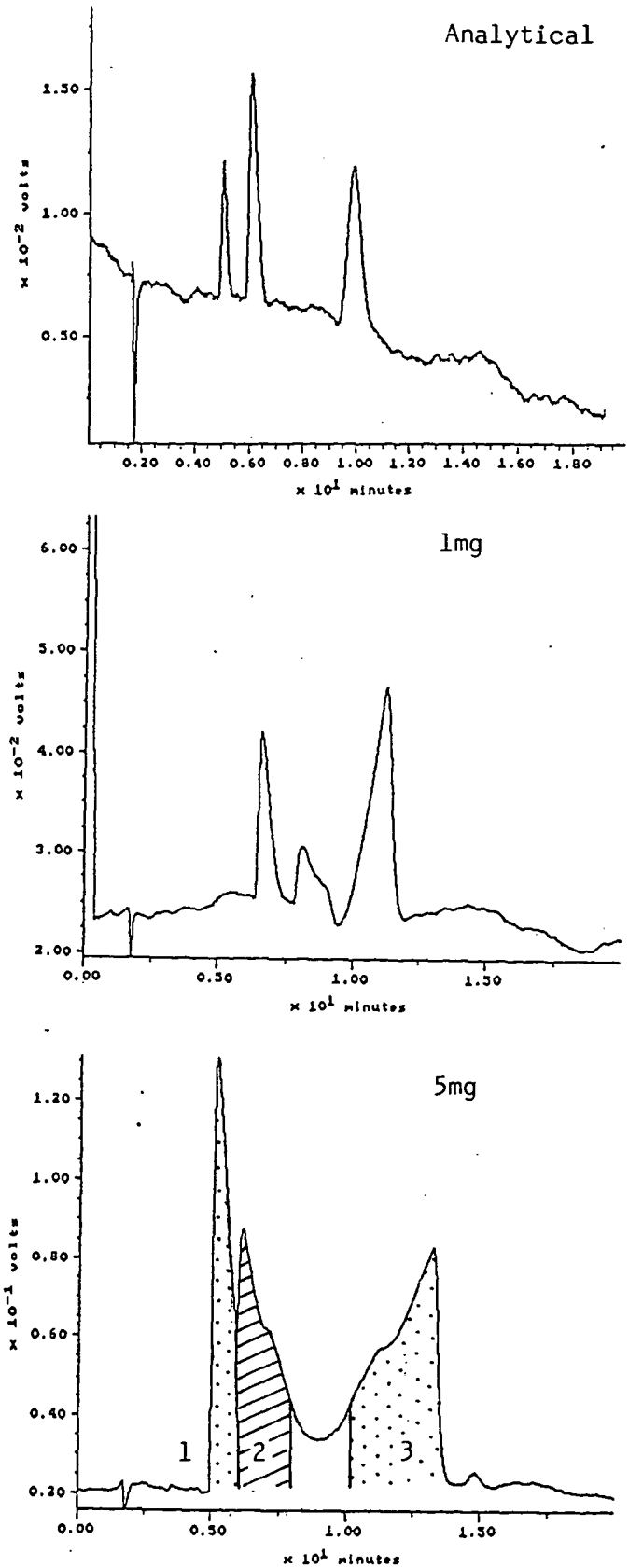


FIGURE IV.2.34

HPLC SEPARATION OF FRACTION IV

Analytical, Preparative and RI traces showing collection points

FIGURE IV.2.35

^{1}H NMR SPECTRUM FOR KESSANE

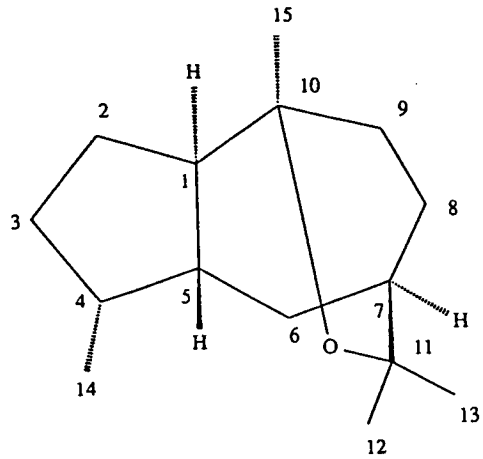
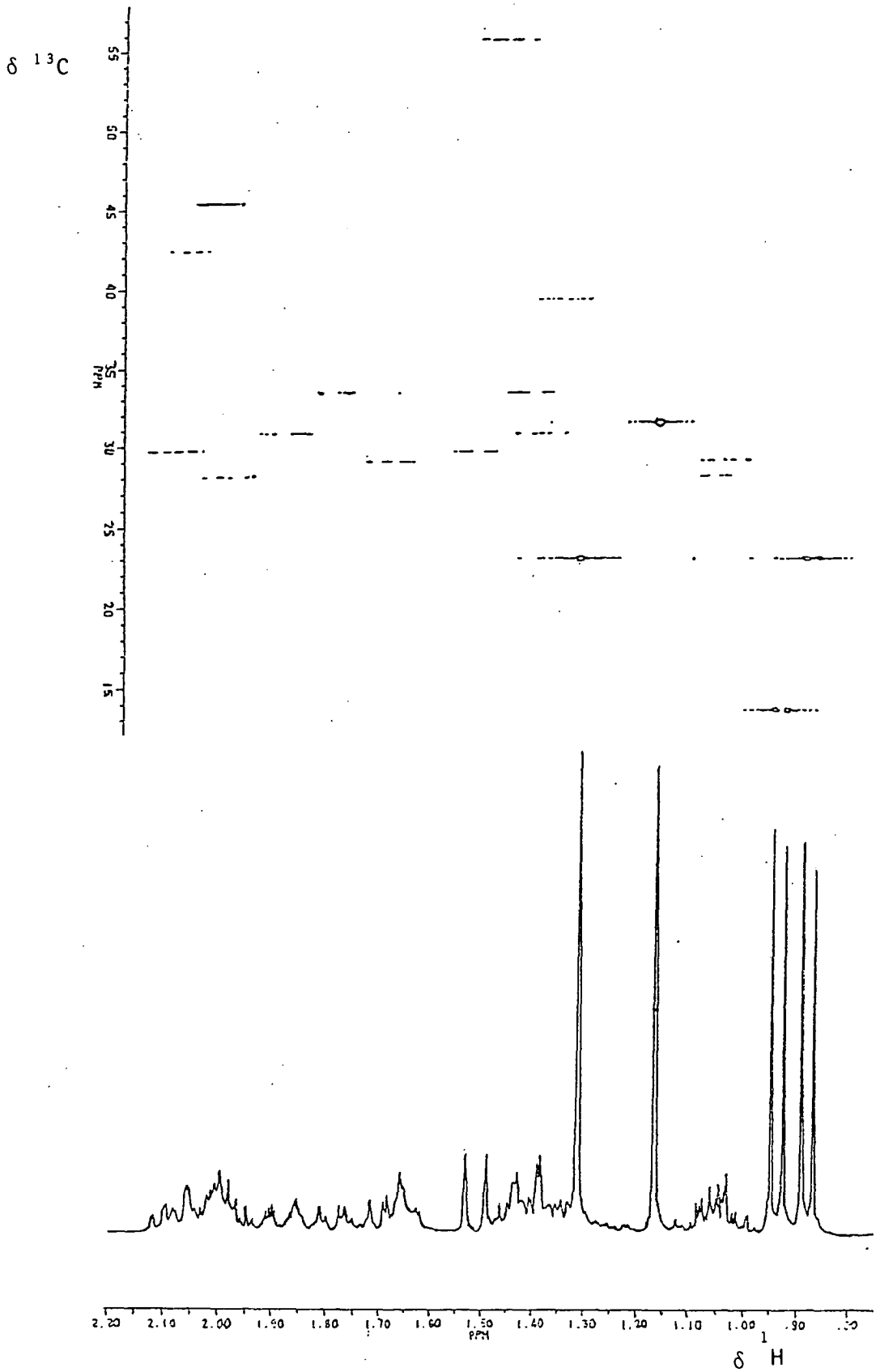
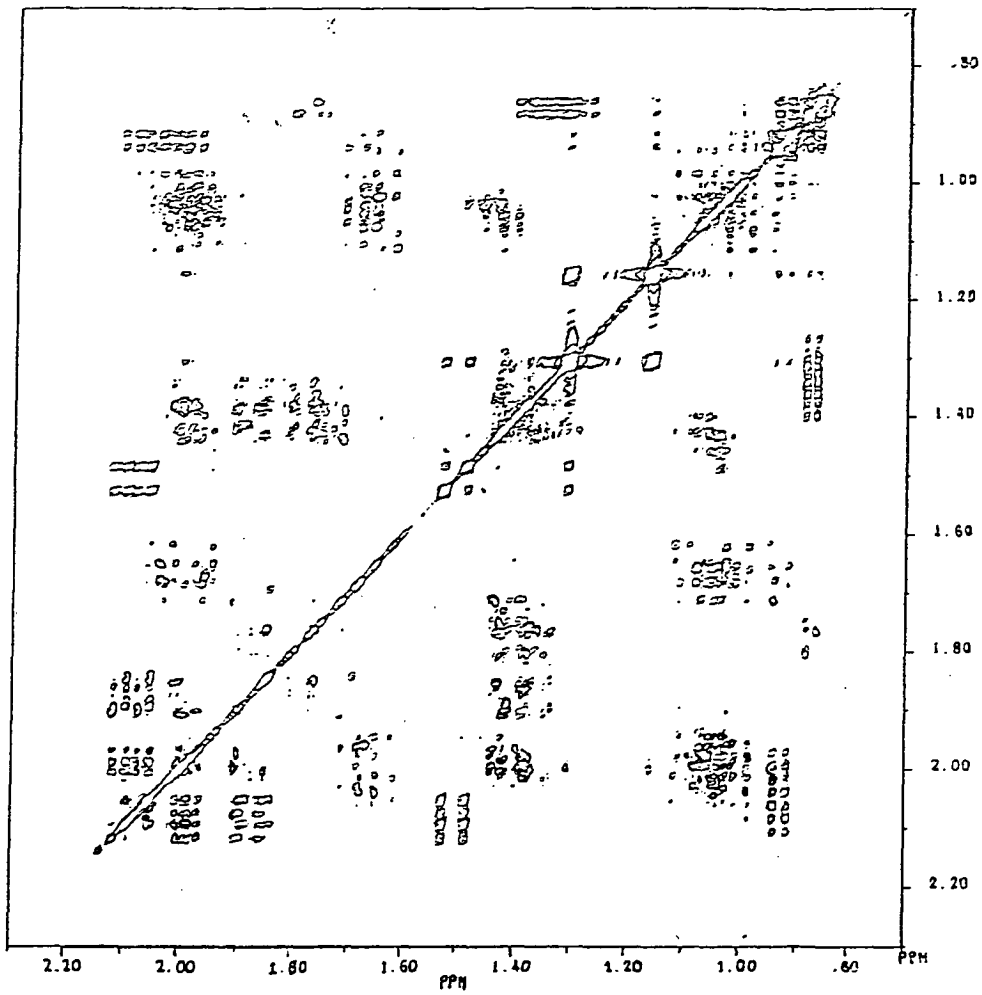


FIGURE IV.2.36



^{1}H-^{13}C CORRELATED 2D NMR (XHCORR)
SHOWING DIRECT (ONE-BOND) CONNECTIVITIES FOR LIGULOXIDE

FIGURE IV.2.37



^{1}H-^{1}H CORRELATED (COSY) SPECTRUM OF LIGULOXIDE

compound is different enough to exclude it as a possible structure. The peaks cited were at 0.95, 1.10, 1.22 and 1.26 ppm.

Kessane has been observed in relatively few plants species, including the grass *Bothriochloa* sp. and Japanese Valerians.

TABLE IV.2.9

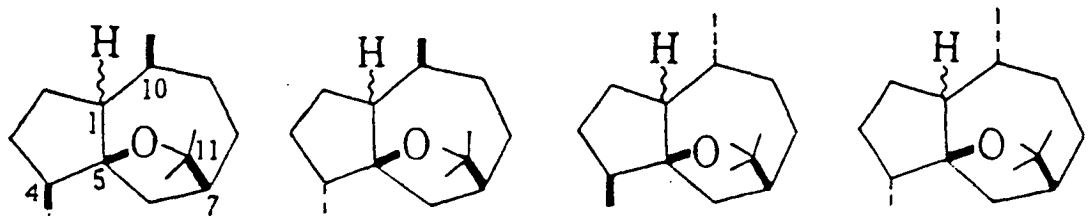
NMR DATA FOR KESSANE ($C_{15}H_{26}O$)

	$\delta^{13}C$		$\delta^{1}H$
C_{11}	74.70	q	
C_{10}	73.96	q	
C_1	50.17	CH	1.67
C_5	41.50	CH	2.00
C_7	35.72	CH	1.67
C_9	34.74	CH_2	1.71, 1.55
C_8	33.28	CH_2	1.85, 1.33
C_4	32.85	CH	2.05
C_3	32.09	CH_2	1.95, 1.06
$C_{12,13}$	31.13	CH_3	1.252
C_{14}	28.39	CH_3	1.116
$C_{12,13}/C_2$	28.24	CH_3/CH_2	1.247, 1.52, 1.37
C_6	24.19	CH_2	2.15, 1.56
C_{15}	18.53	CH_3	0.791

Analysis of the ^{1}H and ^{13}C nmr data for Fraction b (see Figures IV.2.36 and IV.2.37), yielded a very similar partial skeleton as kessane, but it had two methyl doublets in the ^{1}H nmr, indicating that the ether bridge was shifted relative to kessane. See Table IV.2.10. Confirmation of the structure as liguloxide was accomplished by comparison with the ^{1}H nmr (low field) spectra of the eight known diastereomers of guaioxide. Only isomer II (liguloxide) showed a similar spectrum. The structures of the isomers of guaioxide (I) and their ^{1}H nmr data is shown in Figure IV.2.38, and the mass spectrum of liguloxide is given in Figure IV.2.39.

FIGURE IV.2.38

STRUCTURE AND NMR DATA OF ISOMERS OF GUAIOXIDE



I : 1β-H II : 1β-H III : 1β-H IV : 1β-H

V : 1α-H VI : 1α-H VII : 1α-H VIII : 1α-H

I	II	III	IV	V	VI	VII	VIII	FRACTION b
0.88	0.87	0.95	0.85	0.93	0.90d	0.85d	0.87	0.88
0.97	0.93	0.98	0.90	0.93	0.98	0.95	0.89	0.93
1.16	1.16	1.18	1.21	1.15	1.15	1.19	1.18	1.16
1.31	1.32	1.36	1.33	1.35	1.30	1.29	1.24	1.31

(I) guaioxide

(II) 4-epiguaioxide (liguloxide)

(III) 10-epiguaioxide (bulnesoxide)

(IV) 10-epiliguloxide

(V) 1-epiguaioxide

(VI) 1-epiliguloxide

(VII) 1-epi,10-epi-guaioxide

(VIII) 1-epi,10-epi-liguloxide

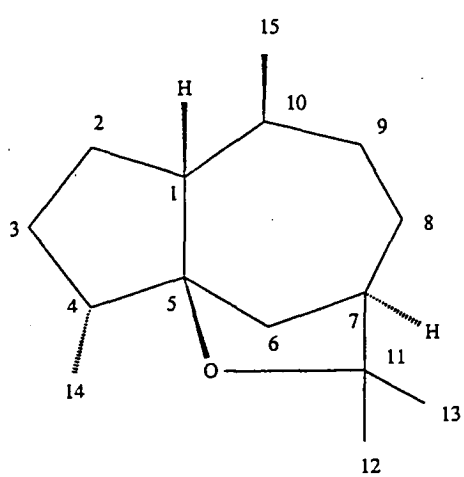
((I)-(VI) Ishii H. *et al.*,1972, (VII-VIII) Hirota H. *et al.*, 1980)

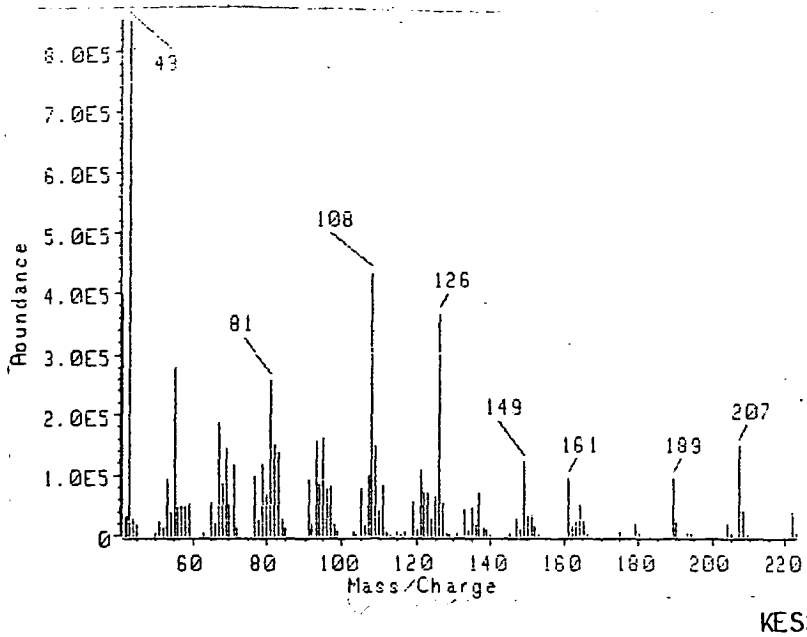
TABLE IV.2.10

NMR DATA OF LIGULOXIDE ($C_{15}H_{26}O$)

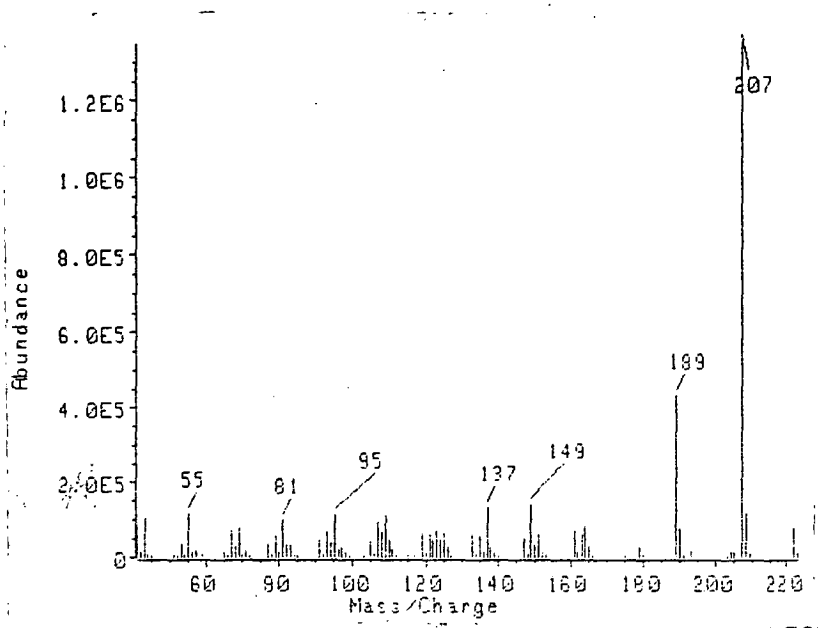
	$\delta\ ^{13}C$		$\delta\ ^{1}H$
C_5	92.57	q	
C_{11}	80.64	q	
C_1	55.78	CH	1.45
C_7	45.35	CH	2.00
C_4	42.32	CH	2.05
C_{10}	39.31	CH	1.34
C_9	33.40	CH_2	1.40, 1.79
$C_{12/13}$	31.43	CH_3	1.162
C_8	30.75	CH_2	1.39, 1.88
C_6	29.63	CH_2	1.51, 2.09
C_3	28.97	CH_2	1.03, 1.68
C_2	27.99	CH_2	1.05, 1.96
$C_{12,13/14}$	22.92	2 x CH_3	1.310, 0.876
C_{15}	13.41	CH_3	0.933

STRUCTURE OF LIGULOXIDE

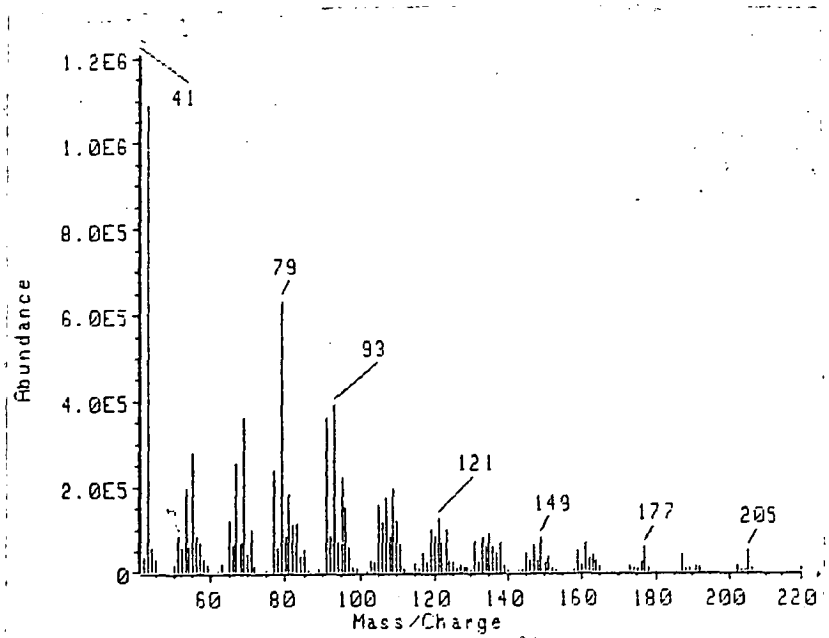




KESSANE



LIGULOXIDE



CARYOPHYLLENE OXIDE

FIGURE IV.2.39

MASS SPECTRA OF ISOLATED COMPONENTS FROM MW OIL

2.10 CONCLUSIONS

From the preceeding work, the following conclusions can be made. The most significant point is that the unique spicey, fruity odour identified by smelling fractions from MW oil is largely due to the presence of one component. This constituent was identified as liguloxide. It it absent from the other oils (by mass spectral analysis).

Other components of the MW oil contributed to the spicey odour. These were found in fractions IIb and IIc. However, the quantities present in the fractions collected only enabled them to be detected organolepticlly, but not to be identified.

The major component of many of the oils was bicyclogermacrene. Germacrene-D was also an important bulk component.

The process of pre-fractionating Olearia essential oil prior to hplc separation was of great utility. It simplified the subsequent fractionation by reducing the number of components present, which may obsure one another.

2.10a AN OVERVIEW OF THE ESSENTIAL OILS OF OLEARIA CLONES

Data from the combination of hplc, gcms and nmr analyses was used to determine the identity of some of the major components in the essential oils from the six Olearia clones.

Thirteen compounds were identified by gc/ms, ftir and nmr amongst the six oils. These were:

1 α-pinene
2 β-pinene
3 1,8-cineole
4 linalool
5 α-terpineol
6 caryophyllene
7 germacrene-D
8 bicyclogermacrene
9 elemol
10 spathulenol
11 γ-eudesmol
12 β-eudesmol
13 α-eudesmol

A comparison of percentage areas from steam distilled oils is shown in Appendix G.

A further three compounds were identified in MW oil by nmr, namely liguloxide, caryophyllene oxide and kessane. In the first instance, the number of components identified by gcms was 19, with limonene, ledol, viridiflorol, globulol, δ-cadinene and γ-elemene being recognised, in addition to the list of 13. The presence of these components was not confirmed by additional investigations, so they have been omitted from this discussion. Of the thirteen remaining, spathulenol, germacrene-D, bicyclogermacrene, caryophyllene, elemol, 1,8-cineole and the three eudesmol isomers were all confirmed by Ftir.

The separation of fractions from MW oil resulted in the isolation of germacrene-D, bicyclogermacrene, caryophyllene oxide, spathulenol, liguloxide and kessane which were confirmed by nmr analysis. Bicyclogermacrene and caryophyllene were isolated and identified from GL oil.

Figures IV.2.5 and IV.2.15 show the traces for all six clone oils from gc and hplc analyses, respectively. The relative differences are clearly visible. The labelling of the hplc traces serves to indicate a) the position of peaks 6, 7 and 8 and b) the general area where peaks 1 to 5 and the eudesmols may occur. The position of the pinenes, 1,8-cineole, linalool, α-terpineol and caryophyllene were determined by the method of peak enhancement with MW oil. The positions of peaks 7 and 8, however, are known from the work on fractionation and purification of MW oil.

The labels for the eudesmols have been placed tentatively after comparison with the trace obtained by running a sample of *Leptospermum lanigerum* oil, which is known to contain significant quantities of these alcohols.

The hplc chromatograms show that amongst the sesquiterpene hydrocarbons, the six oils vary markedly. One obvious feature is the small group of components just preceeding bicyclogermacrene. This group does not occur in the other oils and may be contributing to the exotic fruity, and quite distinct odour of this oil.

The major components of the oils are as follows:

GL	caryophyllene	38%
EP	β-eudesmol	35%
EN	bicyclogermacrene	25%
BU	bicyclogermacrene	34%
PP	linalool	26%
MW	bicyclogermacrene	19%

These figures can only be used as a rough guide, since a variation of as much as 10% has been observed in these components, depending on the time of harvest (see IV.7).

Thus, the techniques used in separation and identification are useful and viable as means of determining the composition of these oils. Chemical characterisation of essential oils is useful in distinguishing one clone from another.

2.10b CHEMOTAXONOMY OF OLEARIA CLONES

The six Olearia clones are all presently classified as *Olearia phlogopappa* (Orchard T., pers. comm.). However, there are obvious morphological differences between them; each can be distinguished from the others, which suggests they may be different enough to give them an individual identity.

An evaluation of essential oils of related species has been used in other cases to aid in the distinction between types, as shown in the literature review. Here also, the oils from the six Olearia clones are markedly different. The gc analyses of the oils have been presented in Figure IV.2.5, and the following distinguishing features can be identified:

Great Lake

Major components - caryophyllene, bicyclogermacrene and spathulenol. There is little eudesmol in this oil, and the monoterpene content is low.

Elephant Pass

Major component - β-eudesmol. The bicyclogermacrene and spathulenol components are present, but not predominant. The monoterpenes comprise mostly α-pinene, but the mixture is complex, including β-pinene and α-terpineol. There is also a large distinguishing peak which occurs just before caryophyllene (retention time = 13.44 min).

Eaglehawk Neck

Major component - bicyclogermacrene. The other major sesquiterpenes are germacrene-D and spathulenol. However, this oil can be distinguished by the occurrence of a large unidentified sesquiterpene which appears after spathulenol and has a retention time of 18.26 min. For all practical purposes, this oil is devoid

of eudesmols.

Buckland

Major components - bicyclogermacrene and spathulenol. This oil is similar to that of EN, except that it lacks both the unidentified sesquiterpene and linalool, which occurs in all of the other oils. It possesses a distinguishing pair of peaks around 14.7 minutes, the latter of which is caryophyllene.

Paradise Plains

Major component - linalool. This is probably the most complex of the oils. It contains a wide variety of monoterpene components. In addition, the three eudesmol isomers are all present in considerable quantities. Of less importance are bicyclogermacrene and caryophyllene. There seems to be an absence of germacrene-D and spathulenol, however, it is the only oil that contains elemol to any significant extent.

Mount Wellington

Major components - bicyclogermacrene, germacrene-D and caryophyllene. This oil also is quite complex, containing a complement of eudesmol isomers and two other major components: liguloxide and kessane, as well as caryophyllene oxide. These components were not found in the other oils by ms comparison. The monoterpenes are present, but not prevalent in this oil.

Each of the oils presents an image of peaks which can be used to fingerprint it. An impression of the overall picture of each chromatogram is presented in diagramatic form in Figure IV.2.13. These patterns are constant for each oil, and can, therefore, be used for identification purposes. It is also apparent from this work that the six clones that were selected are not only morphologically distinguishable, but also contain essential oils that are as individual as the odour impressions they produce.

With respect to bringing Olearia clones into a commercial cropping situation, the composition of the oil is only one of the factors that plays an improtant role. Agronomic characteristics must also be considered. Hence, the six clones were put into a multi-location growth trial.

3. MULTI-LOCATION CLONAL PERFORMANCE TRIALS

The multi-location clonal performance trials, replicated at Bushy Park and Ouse, were set up as detailed in Materials and Methods. The analysis and discussion of the data collected is given below.

3.1 ANALYSIS OF GROWTH DATA

3.1a PRE- AND POST HARVEST DATA FROM BUSHY PARK

Growth increments were calculated as follows. The height at harvest (August '87) was HT14, since this was the 14th month after the trial was initiated. Similarily, D14 and W14 are the stem diameter and width at harvest. The incremental change in height from the 3rd month to the 14th month, that is, nine months growth, is IHT14. ID14 and IW14 are the incremental increases in stem diameter and width, respectively, over a 5 month period prior to harvest. Measurements from the 3rd to 7th months were not available so ID14 = D14 - D8, and IW14 = W14 - W8.

The post-harvest parameters were similarily calculated, where HT21, D21 and W21 were data from the 21st (and last) month of the trial, and IHT21, ID21 and IW21 were (HT21 - HT15), (D21 - D15) and (W21 - W15), respectively. HT15, D15 and W15 were measured the month after harvest, that is, in September '87.

The data was analysed using model 6 of the mixed model least-squares and maximum likelihood computer program written by Harvey (1987). The statistical model used is outlined in Table IV.3.1.

TABLE IV.3.1

EXPECTED MEAN SQUARES FOR ANALYSIS OF VARIANCE AND COVARIANCE OF BUSHY PARK PRE- AND POST HARVEST DATA

The variance component σ_b^2 is due to blocks, σ_{cb}^2 is due to clone x block interaction, σ_c^2 is due to clones, and σ_e^2 is due to error.

SOURCE	d.f.	EXPECTED MEAN SQUARES
Blocks	3	$\sigma_e^2 + k_4 \sigma_{cb}^2 + k_5 \sigma_b^2$
Clones	5	$\sigma_e^2 + k_2 \sigma_b^2 + k_3 \sigma_c^2$
Clones x Blocks	15	$\sigma_e^2 + k_1 \sigma_{cb}^2$
Error	217	σ_e^2

The individual broad sense heritability was calculated as:

$$h_b^2 = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_{cb}^2 + \sigma_e^2}$$

and the heritability of clonal means as:

$$h_{\bar{c}}^2 = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_{cb}^2/(k_3/k_2) + \sigma_e^2/k_3}$$

(Cotterill P.P. and Zed P.G., 1980)

3.1b. MULTILOCATION TRIAL

The characters used in this experiment were HT, D, W, IHT, ID and IW where the first three are height, stem diameter and width in August '87 (the 14th month), and the latter three characters are the increments from November '86 to August '87, (month 14 - month 5).

The multilocation trial was analysed by the GLM procedure of SAS (1987), using the model outlined in Table IV.3.2.

TABLE IV.3.2

EXPECTED MEAN SQUARES FOR MULTILOCATION TRIAL

The variance component $\sigma^2_{bc(s)}$ is due to block x clone within sites, σ^2_{sc} is due to site x clone, σ^2_c is due to clones, $\sigma^2_{b(s)}$ is due to blocks within sites, σ^2_s is due to site and σ^2_e is due to error.

SOURCE	d.f.	EXPECTED MEAN SQUARES
Site	1	$\sigma^2_e + k_{11}\sigma^2_{bc(s)} + k_{12}\sigma^2_{sc} + k_{13}\sigma^2_c + k_{14}\sigma^2_{b(s)} + k_{15}\sigma^2_s$
Block (site)	6	$\sigma^2_e + k_7\sigma^2_{bc(s)} + k_8\sigma^2_{sc} + k_9\sigma^2_{sc} + k_{10}\sigma^2_{b(s)}$
Clone	5	$\sigma^2_e + k_4\sigma^2_{bc(s)} + k_5\sigma^2_{sc} + k_6\sigma^2_c$
Site x Clone	5	$\sigma^2_e + k_2\sigma^2_{bc(s)} + k_3\sigma^2_{sc}$
Block x Clone (Site)	30	$\sigma^2_e + k_1\sigma^2_{bc(s)}$
Error	435	σ^2_e

The significance of each effect was tested using the mean square of the effect containing all other variance components except the component of interest. For instance, the site x clone mean square was the error for testing the significance of the variance component due to clones.

Variance components in the model were estimated using the REML option of the VARCOMP procedure of SAS (1987).

The percentage of the total ($\sigma^2_c + \sigma^2_{bc(s)} + \sigma^2_{b(s)} + \sigma^2_s + \sigma^2_{sc} + \sigma^2_e$) variance attributable to each components was calculated, and

individual broad sense heritability was estimated as :

$$h_b^2 = \frac{\sigma_c^2}{\sigma_e^2 + \sigma_{cb(s)}^2 + \sigma_{sc}^2 + \sigma_e^2}$$

The heritability of clonal means was calculated as:

$$h_{\bar{c}}^2 = \frac{\sigma_c^2}{\sigma_e^2/(NBS) + \sigma_{cb(s)}^2/(BS) + \sigma_{sc}^2/S + \sigma_c^2}$$

where N = the number of replicates / plot = k_4
B = the number of blocks / site = k_5/k_4
S = the number of sites = k_6/k_5

Insufficient computer memory was available to calculate covariance component for estimating genetic correlations from this model. Correlations were therefore calculated using Harvey (1987) using the model indicated in Table IV.3.1. However, in this case the block within site variance was compounded with the variance due to sites (d.f. = 8),

3.2 INFLUENCE OF HARVEST ON CLONAL PERFORMANCE

3.2a GENERAL EFFECTS

Only the data from Bushy Park was used in the study of the effect of harvest on clonal performance, since no harvest occurred at Ouse during the term of this investigation.

The analysis of variance is shown in Table IV.3.3. The results indicate that there is a significant difference between clones for all pre- and post harvest characters, except ID.

The effect of cutting the plants to a uniform height at harvest was to decrease the difference between clones and the heritability of both height and width (Table IV.3.3).

For height, F values fell from 48.5 to 4.9, and the heritability from 0.62 to 0.16. This may be due to several compounded factors including a nursery treatment effect, wherein all clones were not the same size when they were planted out. Thus, a harvest had the effect of reducing the difference in height between

TABLE IV.3.3
ANALYSIS OF VARIANCE
PRE- AND POST HARVEST

	Source	d.f.	Mean Squares	F	Probability	h^2_c
Pre-Harvest						
HT 14	Clone	5	18718.0388	48.453	***	0.617
	Block	3	359.6298	0.932	ns	
	Cl x B	15	385.6995	1.361	ns	
	Error	208	283.3529			
D 14	Clone	5	65.2112	6.616	***	0.202
	Block	3	63.1078	6.403	**	
	Cl x B	15	9.8566	1.908	*	
	Error	208	5.1667			
W 14	Clone	5	4731.0445	24.627	***	0.328
	Block	3	361.3556	1.881	ns	
	Cl x B	15	192.1070	0.805	ns	
	Error	208	238.6042			
IHT 14	Clone	5	18052.5381	50.878	***	0.619
	Block	3	276.3000	0.779	ns	
	Cl x B	15	354.8214	1.299	ns	
	Error	208	273.1781			
ID 14	Clone	5	7.2114	1.858	ns	0.031
	Block	3	22.7485	5.806	**	
	Cl x B	15	3.8820	1.512	ns	
	Error	208	2.5683			
IW 14	Clone	5	1114.4104	8.892	***	0.211
	Block	3	56.0836	0.447	ns	
	Cl x B	15	125.3279	1.357	ns	
	Error	208	95.3884			
Post Harvest						
HT 21	Clone	5	12045.1599	4.893	**	0.156
	Block	3	1326.4872	0.539	ns	
	Cl x B	15	2461.5306	2.024	*	
	Error	208	1215.9663			
D 21	Clone	5	24.7544	8.572	***	0.257
	Block	3	0.2098	0.073	ns	
	Bl x C	15	2.8878	1.933	*	
	Error	208	1.4938			
W 21	Clone	5	2089.4415	6.605	***	0.231
	Block	3	94.8469	0.300	ns	
	Cl x B	15	316.3395	2.357	**	
	Error	208	134.1883			

the clones.

In addition, there was a significant block effect in the pre-harvest situation, which disappeared by the post-harvest stage. This was probably due to localised variations in watering, or a carry over effect from the nursery. The strongest plants may have been placed together when planting out.

3.2b CORRELATIONS AND CLONAL RANKING

Table IV.3.4 shows phenotypic correlations in the upper right and genotypic correlations with standard errors in the lower left, for traits at Bushy Park.

The genotypic correlations amongst characters were all high and positive (Table IV.3.4), suggesting that height, width and stem diameter all basically reflect the same character of 'plant size', and that one character would more or less suffice to describe clone vigour. There are strong genetic correlations between pre- and post harvest measures indicating that while the degree of difference between clones was reduced after harvest (Table IV.3.3), the rank order of the clones was similar (see Table IV.3.7). For example, clones 5 and 6 were the most vigorous (based on HT, W and D), both before and after harvest (Table IV.3.7).

Table IV.3.4 also shows that the phenotypic correlations between all the characters examined is positive. The strongest correlation, of 0.996, exits between the height and the increase in height. Of the other parameters, height is most closely correlated with width.

Means in Table IV.3.7 were calculated using LSDMOD option of the ONE WAY procedure of the SPSS-X 2.1 program, and clones which are significantly different are indicated with an asterisk ($p < 0.05$). The variables were HT 10, D 10, W 10, IHT 10, ID 10 and IW 10 for Ouse; HT 14, D 14, W 14, IHT 14, ID 14, IW 14, HT 21, D 21, W 21, IHT 21, ID 21 and IW 21 for Bushy Park. The 10 and 14 series represent the situation before harvest, and the 21 series of data came from measurements after harvest.

TABLE IV.3.4

PRE- AND POST HARVEST

Genotypic correlations with standard errors (below the diagonal), and phenotypic correlations (above the diagonal), for traits at Bushy Park.

	HT 14	D 14	W 14	IHT 14	ID 14	IW 14	HT 21	D 21	IW 21
HT 14		0.522	0.685	0.996	0.252	0.476	0.387	0.348	0.630
D 14	0.900 ± .106		0.349	0.502	0.739	0.250	0.135	0.262	0.304
W 14	0.925 ± .014	0.932 ± .093		0.676	0.121	0.677	0.314	0.278	0.695
IHT 14	0.999 ± .001	0.877 ± .125	0.917 ± .081		0.245	0.474	0.396	0.327	0.628
ID 14	0.859 ± .317	0.903 ± .207	0.741 ± .401	0.854 ± .320		0.133	0.042	0.079	0.116
IW 14	0.906 ± .102	0.936 ± .105	1.038 ± .026	0.898 ± .109	0.779 ± .389		0.341	0.232	0.342
HT 21	1.017 ± .038	1.025 ± .069	0.810 ± .193	1.027 ± .035	1.593 ± .610	0.876 ± .151		0.105	0.390
D 21	0.729 ± .229	1.094 ± .065	0.799 ± .190	0.692 ± .252	1.233 ± .282	0.910 ± .126	0.667 ± .300		0.314
W 21	0.961 ± .051	1.170 ± .135	0.909 ± .092	0.951 ± .059	1.595 ± .621	1.083 ± .045	0.997 ± .057	0.871 ± .143	

3.3 MULTILOCATION TRIAL

3.3a GENERAL EFFECTS

The analysis of variance for the multilocation trial is presented in Table IV.3.5. With the exception of DIA, there is a significant difference between sites, probably due to a variety of factors including different planting times.

The clone effect was only significant for DIA ($p \leq 0.01$) and IHT ($p \leq 0.05$).

Most importantly, the site x clone interaction was highly significant ($p \leq 0.01$) for all characters except DIA (Table IV.3.5), and even within sites, the block x clone effect was highly significant ($p \leq 0.001$) for the two diameter measurements. (DIA and IDIA).

These results indicate that the pre-harvest performance of the clones was markedly affected by the site of growth.

3.3b HERITABILITIES AND CORRELATIONS

The heritabilities for the multilocation trials are shown in Table IV.3.5, and genotypic correlations and phenotypic correlations are given in Table IV.3.6. The broad sense individual heritabilities are all comparatively low when compared to estimates based on the analysis of the single site at Bushy Park (Table IV.3.3). This is no doubt due to the inclusion of the relatively large site x clone interaction component in the multilocation trial (Table IV.3.5). Nevertheless, the heritability of clonal means was greater than 0.5 for all characters except IDIA.

As with the harvest analysis, all genotypic correlations were high and positive (Table IV.3.6), with low standard errors. In the commercial situation, this implies that large plants will produce the greatest increases in height, stem diameter and width.

All phenotypic correlations were positive, with the highest correlation between height and the increase in height.

TABLE IV.3.5

MULTILOCATION TRIAL ANALYSIS OF VARIANCE

F Values, Probabilities (ns : not significant, * : $p \leq 0.05$, ** : $p \leq 0.01$, *** : $p \leq 0.001$), and *variance* %.

SOURCE	d.f.	HT	DIA	WIDTH	IHT	IDIA	IWIDTH
Site	1	739.5 ***	4.0 n.s	204.5 ***	47.1 ***	282.3 ***	167.8 ***
Block(Site)	6	0.0 n.s.	0.3 n.s.	0.1 n.s.	0.0 n.s.	0.1 n.s.	0.3 n.s.
Clone	5	3.0 n.s.	13.0 **	2.0 n.s.	9.6 *	1.5 n.s.	4.3 n.s.
Site x Clone	5	15.1 ***	0.9 n.s.	6.7 ***	5.5 ***	5.3 **	5.2 **
Block x Clone(Site)	30	350.6 *	11.7 ***	1.4 n.s.	1.5 *	2.7 ***	1.1 n.s.
Error (within plots)	435						
	h_b^2 =	0.308	0.217	0.105	0.459	0.062	0.176
	h_c^2 =	0.686	0.912	0.521	0.890	0.320	0.749

TABLE IV.3.6
MULTILOCATION TRIAL

Genotypic correlations with standard errors (below the diagonal), and phenotypic correlations (above the diagonal), between traits measured in the multilocation trial.

	HEIGHT	DIAMETER	WIDTH	IHEIGHT	IDIAMETER	IWIDTH
HEIGHT		0.521	0.704	0.851	0.207	0.368
DIAMETER	0.940 ± .065		0.407	0.485	0.679	0.333
WIDTH	0.908 ± .087	0.890 ± .112		0.597	0.170	0.515
IHEIGHT	1.008 ± .006	0.959 ± .048	0.981 ± .029		0.335	0.525
IDIAMETER	1.017 ± .028	1.061 ± .047	1.051 ± .036	0.992 ± .036		0.420
IWIDTH	0.882 ± .113	0.965 ± .053	1.049 ± .035	0.909 ± .089	0.966 ± .057	

3.3c CLONAL RANKING

The clones were re-labelled for ranking as follows:

1 GL
2 PP
3 EP
4 MW
5 BU and
6 EN

At Ouse, the rankings of clones with respect to all three characters, height, stem diameter and width, was similar. Some small deviations exist, however, such as the position of clone 4 in the width ranking. This highlights the shape of the canopy of this clone, which tends to increase in width as readily as height.

In addition, the rankings of clones with respect to increases in the three characters, shows that although 1 is the shortest clone, it is 3 that has the slowest growth rate. These findings are reflected in the Bushy Park width data.

The situation immediately after harvest is similar to pre-harvest. The diameter character shows that the effect of harvest on clone 4 was to increase its ranking, giving it the greatest increase in stem diameter, more so than any of the other clones.

In a broad sense, rank order of the clones remained reasonably similar at both sites (Table IV.3.7), with Buckland (5) and Eaglehawk Neck (6) performing best. However, their relative order changes with site. Clone 6 tended to perform significantly better than clone 5 at Bushy Park for HT, but the reverse was true at Ouse (Table IV.3.7).

3.3d SEASONAL VARIATION IN CLONAL GROWTH RATES

The performance of clones at Ouse and Bushy Park, with respect to relative increases in height, stem diameter and width are shown in Figures IV.3.1, IV.3.2 and IV.3.3 respectively.

All clones reflect the same basic pattern of relative height increase. Increases are greatest during the summer months, and fall to very low levels in the winter. The width increases observed at Ouse during the summer are almost five times those observed at Bushy

TABLE IV.3.7

CLONAL RANKING AT OUSE AND BUSHY PARK

OUSE

HT 10

Mean	Clone	1 3 4 2 6 5
215.25	1	
330.50	3	*
345.25	4	*
354.10	2	*
361.62	6	*
585.90	5	* * * * *

D 10

Mean	Clone	1 2 3 4 6 5
35.02	1	
41.54	2	
48.07	3	
58.23	4	* *
74.08	6	* * * *
77.21	5	* * * *

W 10

Mean	Clone	1 4 3 2 6 5
197.25	1	
276.75	4	*
310.25	3	*
312.56	2	*
363.42	6	* *
376.05	5	* *

IHT 10

Mean	Clone	3 1 4 2 6 5
465.26	3	
514.79	1	
566.05	4	*
581.51	2	*
755.61	6	* * * *
885.16	5	* * * * *

ID 10

Mean	Clone	2 1 3 4 5 6
54.83	2	
55.19	1	
65.76	3	
109.92	4	* * *
121.79	5	* * *
140.74	6	* * *

IW 10

Mean	Clone	1 2 4 3 5 6
493.08	1	
514.69	2	
574.16	4	
574.65	3	
672.12	5	* * * *
782.29	6	* * * * *

BUSHY PARK

HT 14

Mean	Clone	1 3 4 2 5 6
536.75	1	
708.25	3	*
759.25	4	*
760.75	2	*
994.36	5	* * * *
1146.25	6	* * * * *

D 14

Mean	Clone	2 3 1 4 5 6
57.43	2	
60.90	3	
64.17	1	
70.42	4	
79.64	5	* *
91.52	6	* * * *

W 14

Mean	Clone	1 3 2 5 4 6
510.50	1	
563.00	3	
606.00	2	
629.23	5	*
647.75	4	*
630.50	6	* * * * *

IHT 14

Mean	Clone	1 3 4 2 5 6
435.75	1	
606.00	3	*
664.88	4	*
684.38	2	*
904.36	5	* * * *
1032.38	6	* * * * *

ID 14

Mean	Clone	2 3 1 4 6 5
16.58	2	
18.43	3	
20.90	1	
24.88	4	
27.58	6	
29.90	5	* *

IW 14

Mean	Clone	3 1 2 5 4 6
139.25	3	
153.65	1	
189.25	2	
193.33	5	
204.00	4	
289.25	6	* * * * *

HT 21

Mean	Clone	3 1 4 2 6 5
702.00	3	
735.75	1	
846.50	4	
893.50	2	
1083.75	6	* * * *
1143.24	5	* * * *

D 21

Mean	Clone	2 3 1 5 4 6
22.90	2	
31.08	3	*
32.33	1	*
36.65	5	*
37.60	4	*
46.41	6	* * * * *

W 21

Mean	Clone	3 1 2 4 5 6
662.00	3	
693.00	1	
693.00	2	
757.25	4	* * *
807.84	5	* * *
845.00	6	* * * *

IHT 21

Mean	Clone	3 1 4 2 6 5
388.00	3	
500.50	1	
557.00	4	
587.75	2	
688.50	6	*
759.46	5	* *

ID 21

Mean	Clone	2 3 1 6 5 4
9.73	2	
14.15	3	
16.03	1	
21.36	6	*
21.40	5	*
21.65	4	*

IW 21

Mean	Clone	3 2 1 5 4 6
269.00	3	
335.75	2	*
353.25	1	*
363.79	5	*
386.75	4	*
396.25	6	*

1 = G1
2 = PP
3 = EP
4 = MW
5 = BU
6 = EN

Significantly different clones are indicated *. ($p \leq 0.05$)

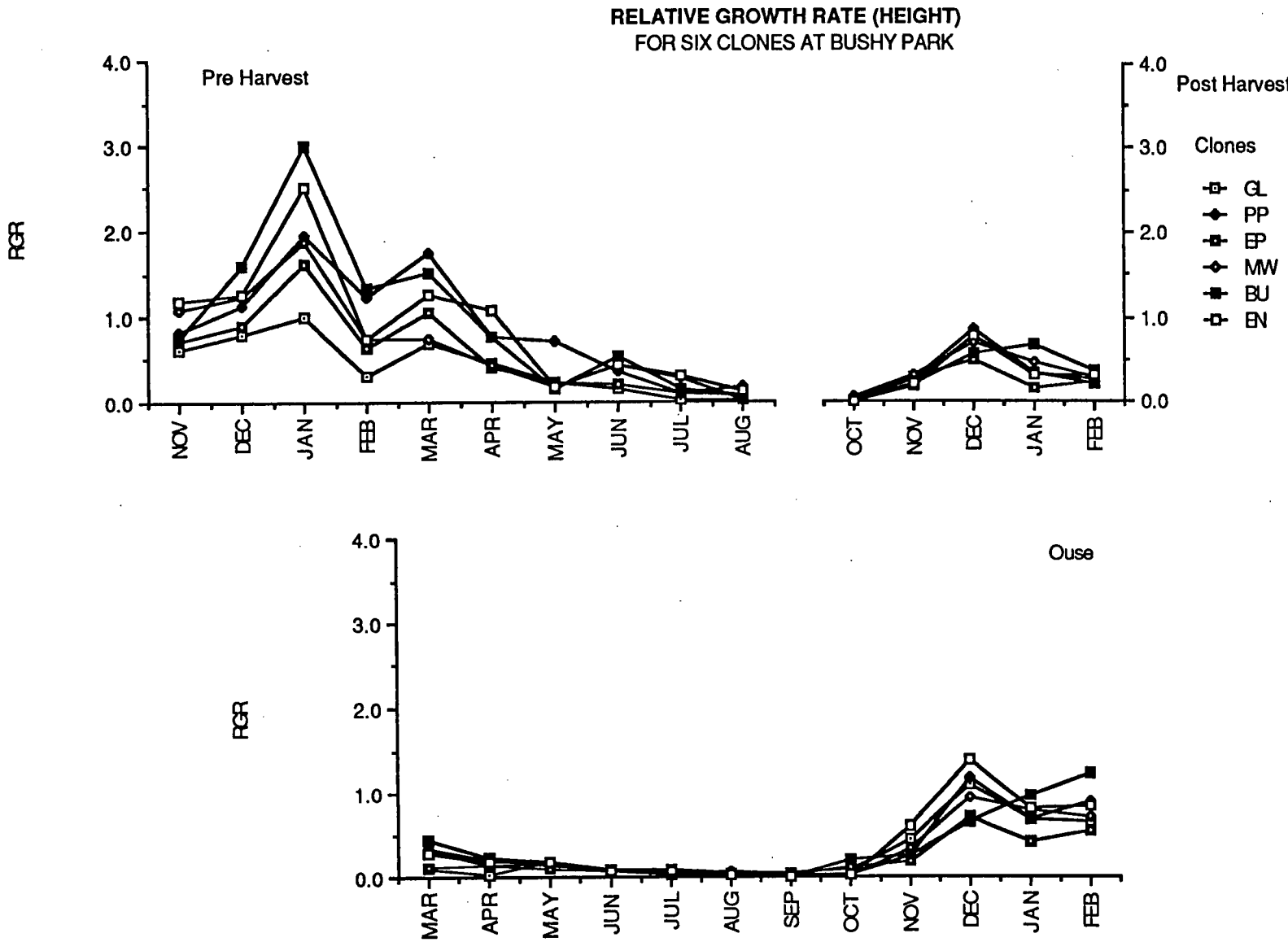
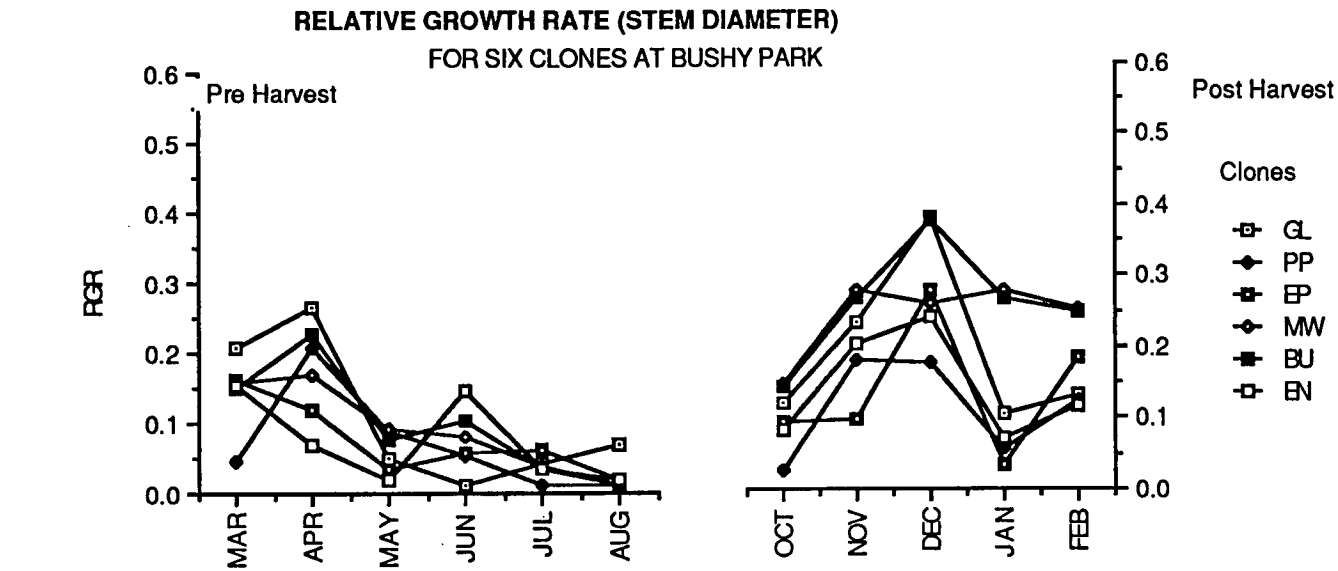

RELATIVE GROWTH RATE (HEIGHT)
FOR SIX CLONES AT BUSHY PARK
Pre Harvest
Post Harvest
Ouse
RGR
Clones
GL
PP
EP
MW
BU
EN
4.0
3.0
2.0
1.0
0.0
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT

FIGURE IV.3.1


RELATIVE GROWTH RATE (STEM DIAMETER)
FOR SIX CLONES AT BUSHY PARK
Pre Harvest
Post Harvest
RGR
Clones
GL
PP
EP
MW
BU
EN
MAR
APR
MAY
JUN
JUL
AUG
OCT
NOV
DEC
JAN
FEB

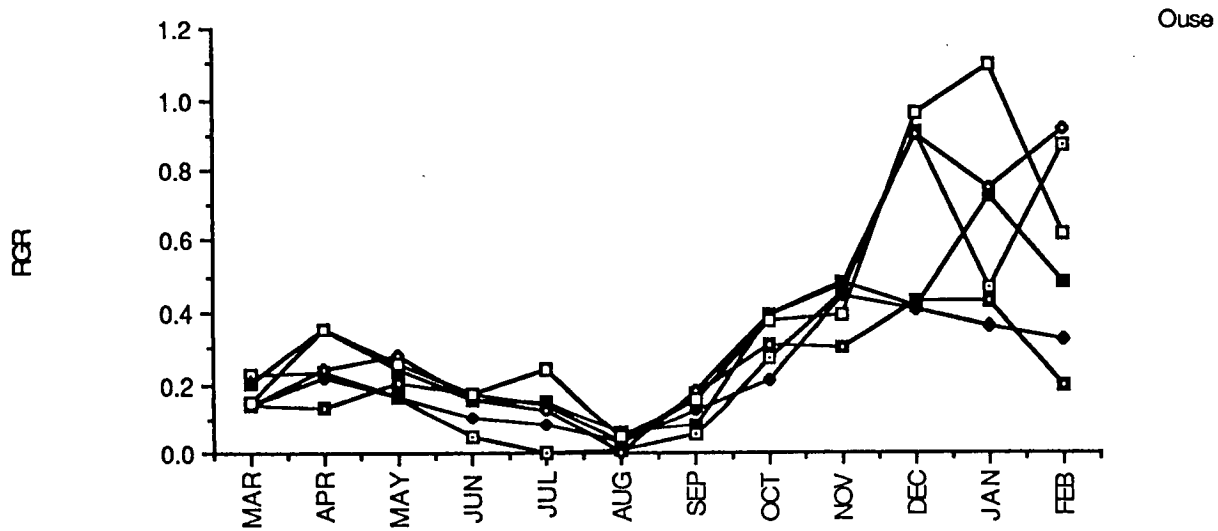

Ouse
RGR
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB

FIGURE IV.3.2

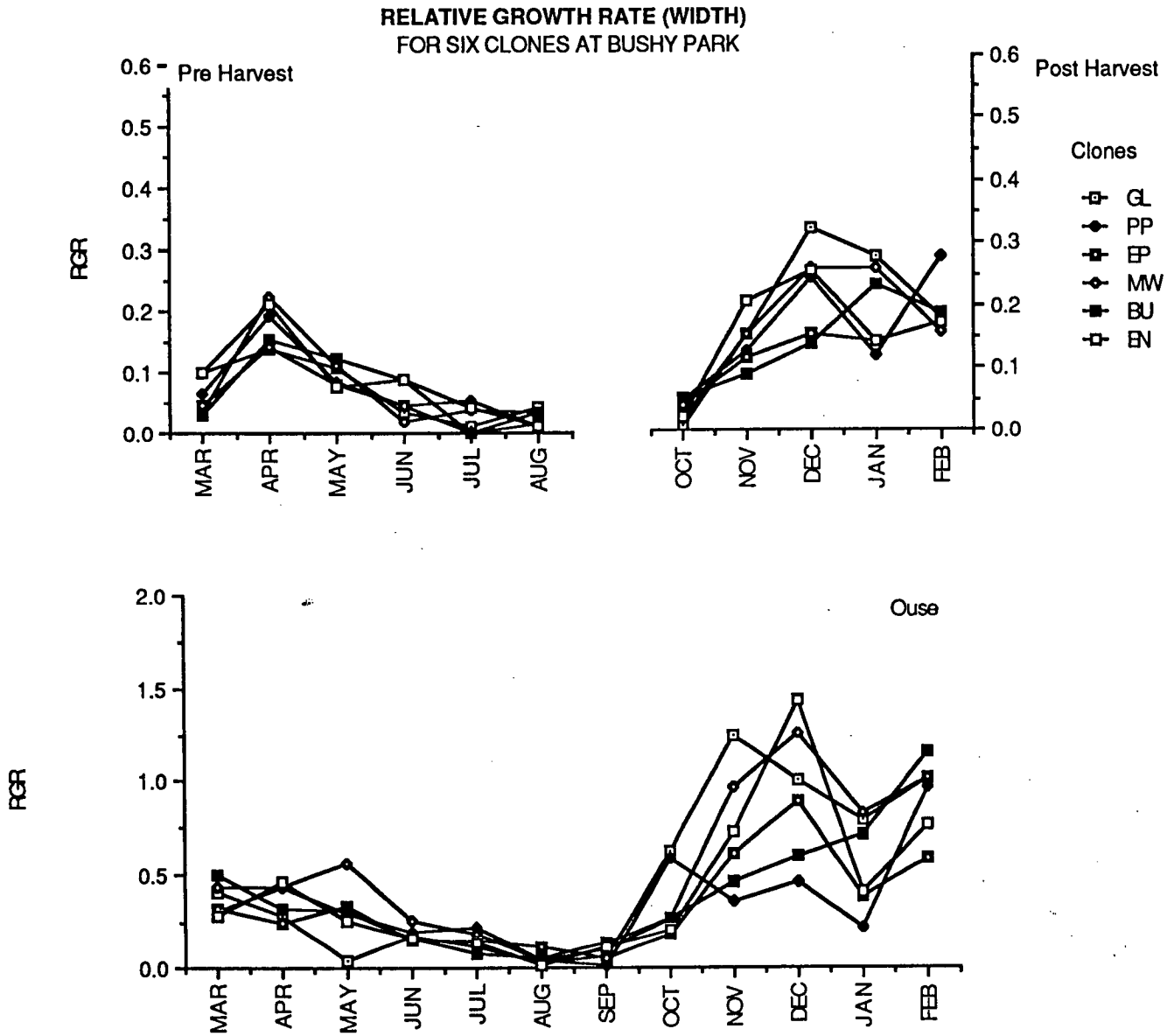

RELATIVE GROWTH RATE (WIDTH)
FOR SIX CLONES AT BUSHY PARK
Pre Harvest
Post Harvest
RGR
Clones
GL
PP
EP
MW
BU
EN
MAR
APR
MAY
JUN
JUL
AUG
OCT
NOV
DEC
JAN
FEB
Ouse
SEP

FIGURE IV.3.3

Park. The slower establishment of the Ouse site meant that during the growing period there was still great scope for increases in width to occur. On the other hand, Bushy Park had already had a summmer growth period (the previous year), and the possibility of expanding laterally had been reduced. In addition, harvesting removed the majority of top growth, but did not decrease the number of plant stems. Hence, there is less potential for width increases to occur.

The stem diameter character has a growth pattern which behaves similarly to that of width. The relative growth rate of stem diameter at Ouse in summer is almost three times that of the same character at that time at Bushy Park.

3.4a VIGOUR IN RELATION TO ALTITUDE OF CLONAL SOURCE PLANTS

Figure IV.3.4 shows the relationship between the altitude at which clonal source plants were located, and the vigour of derived ramets in terms of height increase in December at Ouse and Bushy Park. (Height was used as the best character to describe vigour). The trends observed in Figure IV.3.4 may be interpreted in the following way. Firstly, the large height increase by plants which came from sea level (that is, Eaglehawk Neck), is the result of taking plants from a purely sandy growth medium, to the richer soils at both Ouse and Bushy Park. In addition, both trial areas were irrigated, which would definitely be a contributing factor to the rapid growth of the EN clone.

Secondly, the latter part of the curves on the two graphs in Figure IV.3.4, represents a trends towards a gradual increase in height increment with increasing altitude. Thus, the greater the original altitude, the greater the response when that plant is brought into cultivation at low altitude, with adequate irrigation.

In addition, there seems to be an underlying trend for plants at Bushy Park to have a consistently lower height increase. However, this is probably an effect of the harvest that took place in August.

There is no consistent association between altitude and vigour, except that clones which were moved from extremes of altitude sites were more vigorous when cultivated under the trial conditions. Changes from either low or high altitudes result in an increase in vigour, however, it remains to be determined whether these improvements in performance are due to altitude effects alone, or are influenced by soil type, moisture availability, shelter and so on.

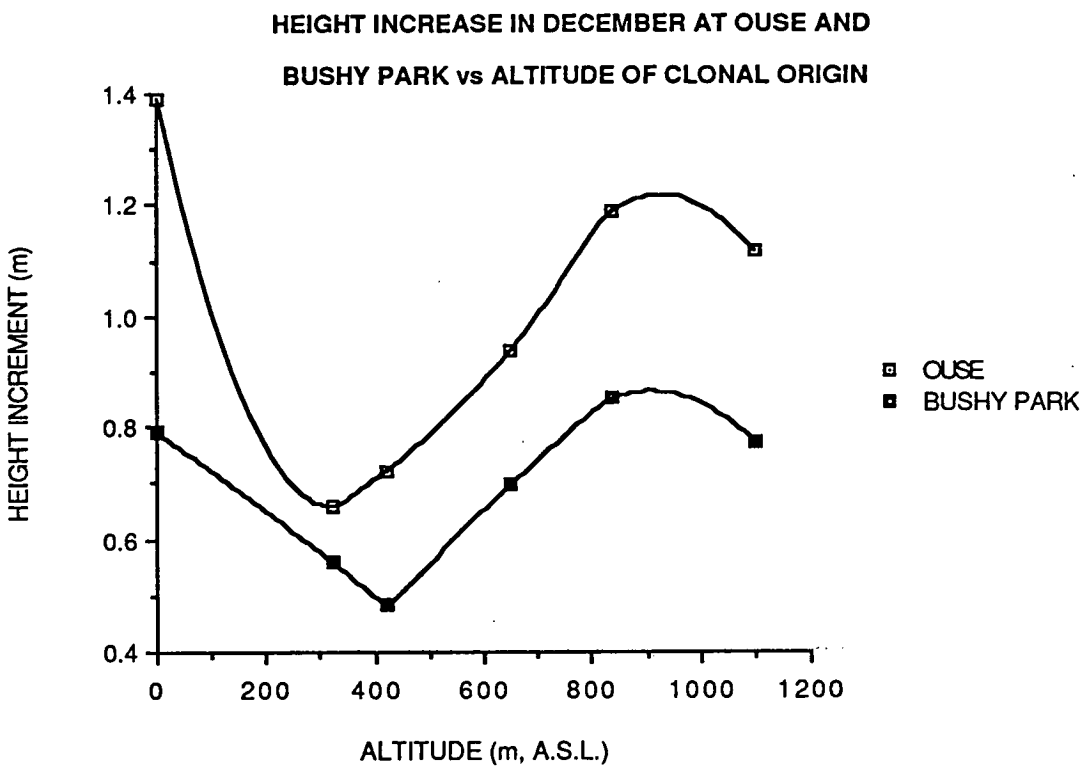

HEIGHT INCREASE IN DECEMBER AT OUSE AND
BUSHY PARK vs ALTITUDE OF CLONAL ORIGIN
HEIGHT INCREMENT (m)
1.4
1.2
1.0
0.8
0.6
0.4
0
200
400
600
800
1000
1200
ALTITUDE (m, A.S.L.)
OUSE
BUSHY PARK

FIGURE IV.3.4

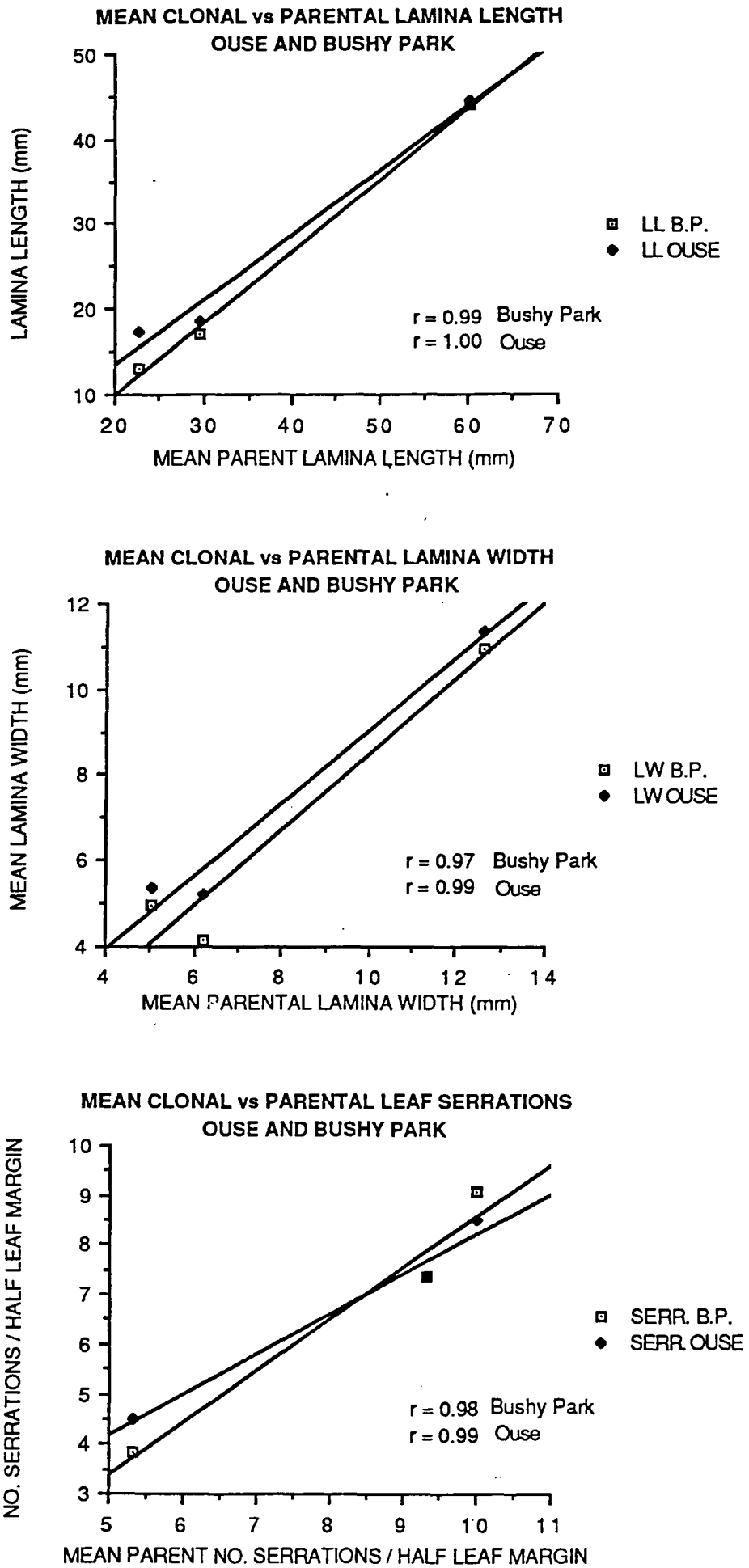
3.4 EFFECT OF GROWING SITE ON MORPHOLOGY OF CLONES

At both the Ouse and Bushy Park sites, the correlation between clonal leaf measurments and values from the original plant (ortet) was scrutinised. Leaves were collected at random from Elephant Pass, Great Lake and Paradise Plains plants grown at the trial sites. The count, means and standard error data are given in Appendices 3, 4 and 5 for Elephant Pass, Great Lake and Paradise Plains, respectively.

Figure IV.3.5 shows the relationship between the mean lamina length, lamina width and laminal serrations compared to the ortet values.

The correlations in all cases are high, ranging from 0.97 to 1.00, which indicates that there is a genetic component to the natural variation in these characters.

FIGURE IV.3.5


MEAN CLONAL vs PARENTAL LAMINA LENGTH
OUSE AND BUSHY PARK
LAMINA LENGTH (mm)
MEAN PARENT LAMINA LENGTH (mm)
LL B.P.
LL OUSE
r = 0.99 Bushy Park
r = 1.00 Ouse
MEAN CLONAL vs PARENTAL LAMINA WIDTH
OUSE AND BUSHY PARK
MEAN LAMINA WIDTH (mm)
MEAN PARENTAL LAMINA WIDTH (mm)
LW B.P.
LW OUSE
r = 0.97 Bushy Park
r = 0.99 Ouse
MEAN CLONAL vs PARENTAL LEAF SERRATIONS
OUSE AND BUSHY PARK
NO. SERRATIONS / HALF LEAF MARGIN
MEAN PARENT NO. SERRATIONS / HALF LEAF MARGIN
SERR. B.P.
SERR. OUSE
r = 0.98 Bushy Park
r = 0.99 Ouse

2.5 MULTI-LOCATION TRIAL - GREAT LAKE SITE

Initially, the site at Stonehut, near Great Lake, was to be the third location of a three-part multi-location trial, along with Bushy Park and Ouse.

The elevation of the site is 1070 m.

The site was prepared and set out similarly to the Ouse and Bushy Park sites. Plants were put in place 17th September, 1986. However, by 4th November, 1986, the decision to replant was made, since there had been heavy losses of plants due to unseasonal snow falls.

A more easily accessible site was found at Wihareja, where the plot was re-established. Strips of ground were sprayed with Roundup where the plants were to be placed, in order to remove the grass which covered the area. No effort was made to remove the rocks which occur throughout the soil. The usual triangular 0.5 m spacing was used when the ramets were planted at the end of February, 1987.

After a period of three months the plot was inspected, and the results were disappointing. Many of the plants had died, presumably due to water stress, arising from a combination of lack of regular watering and root systems that had not become vigorous enough to glean the necessary moisture from the soil. The water stress situation was not mediated by the position of the site, which remained exposed to prevailing winds despite the provision of netting along the fence line.

At the time of last inspection, 17th May, 1988, the following observations were made.

1. The number of surviving plants from 40 of each of the six clones was:

PP	14
GL	3
BU	2
EN	0
EP	0
MW	1

Guards 29 of 38

A typical example of those plants which survived at the Great Lake trial site

PLATE 6

2. On the plants that were not dead, all growth was observed to be re-growth. That is, the original laterals had not survived, but growth had started at the base of the plant. See Plate 6.

3. A harsh winter had occurred immediately after planting. Most plants had not established strong root systems by that time. Those that had were unable to make a solid interaction with the stoney soil. A long, and very dry summer followed, with unseasonal snow at the beginning of March, 1988.

4. The guard plants were of the Great Lake type, and had been more advanced plants than the trial ones. Hence, it seems that they had more rootlets to cope with the added stress of transplantation and establishment. They also had a greater number of stems on which the buds for lateral growth are found.

5. The slightly higher survival rate of the Paradise Plains clone may also be attributed to the fact that they were older cuttings than the other five clones.

4. ASPECTS OF CLONAL GROWTH AND CULTIVATION

4.1a RELATIONSHIP BETWEEN TOTAL DRY MATTER AND MEASURED MORPHOLOGICAL CHARACTERS

A small-scale investigatory trial was run in order to confirm the suitability of using such measurements as height, diameter of stem and plant width as monitors of growth, or more specifically, dry matter production.

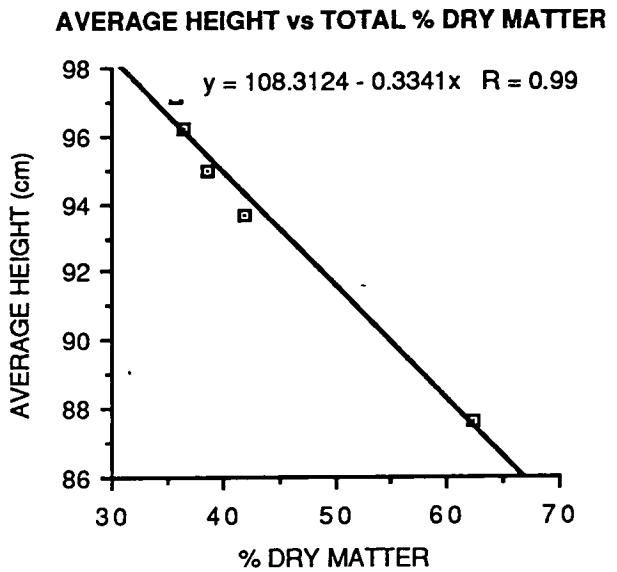
Plants of the Elephant Pass type were chosen due to their upright, vigorous habit. A total of 40 plants were available. Twenty of these were retained for measuring height, stem diameter and width. The other twenty were destructively harvested over a period of four months. Each plant that was cut was considered to be a replicate. Four replicates were possible with five harvest dates. When cutting, the plants were removed down to ground level, and percentage dry matter determinations were carried out. The plants were collected on:

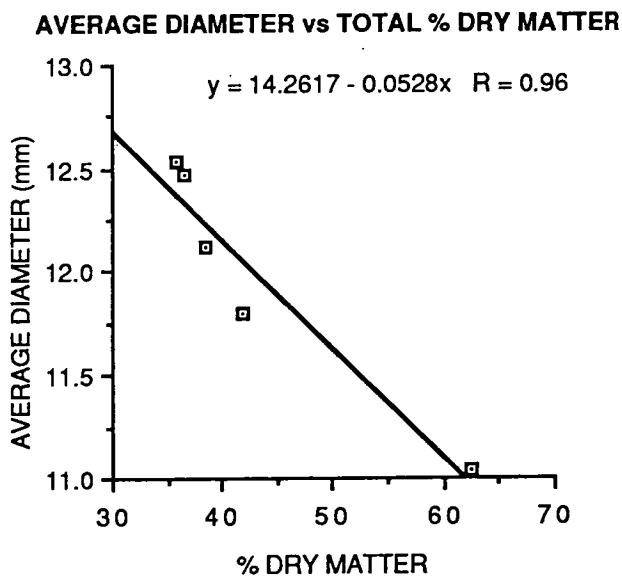
2.3.'88
4.4.'88
22.4.'88
4.5.'88
2.7.'88

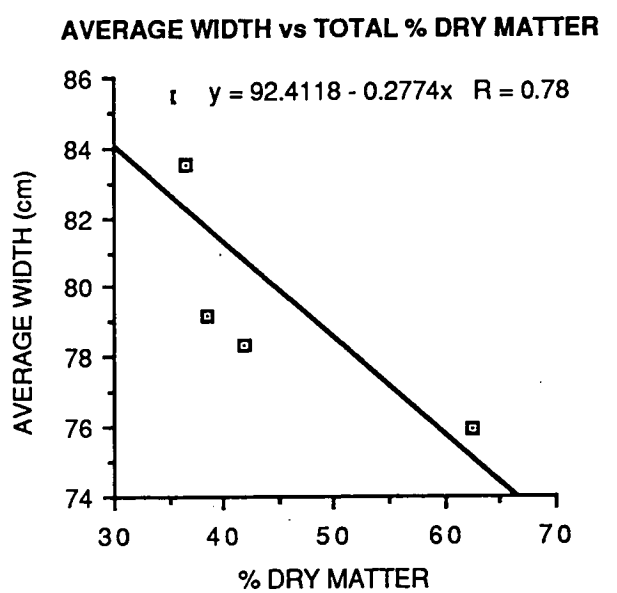
The total percentage dry matter was plotted against the height, stem diameter and width. Figure IV.4.1 shows that height gives an excellent correlation with percentage dry matter ($r = 0.99$), followed by diameter, and width giving the poorest correlation. Plant width is undoubtedly influenced by factors such as proximity to other plants, as well as genetically pre-determined habit and canopy shape. Under normal circumstances, once 100% cover is achieved, no further increase in plant width is possible in the direction of the neighbouring plants. Hence, the growth curve for width versus percentage dry matter is asymptotic, and further increases in dry matter do not result in increases in width.

FIGURE IV.4.1

RELATIONSHIP BETWEEN DRY MATTER AND MORPHOLOGICAL CHARACTERS







4.1b CLONAL DRY MATTER VARIATION

A trial was also instigated, whereby samples were collected for the determination of percentage dry matter in conjunction with percentage essential oil yield estimations. Material was randomly chosen from each of the six clones.

The dry matter content of each clone was determined and calculated as outlined in Materials and Methods. This procedure was carried out monthly at two locations; Ouse and Bushy Park. The percentage dry matter data from September '87 to August '88 at both Ouse and Bushy Park are given in Table IV.4.1 below:

TABLE IV.4.1

PERCENTAGE DRY MATTER FIGURES FOR SIX OLEARIA CLONES AT OUSE AND BUSHY PARK

Date	1987 Sep	Oct	Nov	Dec	1988 Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	MEAN	S.D.
BUSHY PARK														
EP	32.09	58.91	65.17	64.26	44.28	58.45	53.82	61.39	58.79	62.16	60.72	54.47	55.97	8.10
EN	43.00	59.28	66.53	69.98	58.06	59.36	56.61	59.00	64.54	60.98	56.22	51.81	58.43	5.82
BU	57.80	65.99	62.98	67.17	52.66	58.62	53.87	58.52	58.19	58.92	53.75	49.32	57.06	4.83
PP	36.08	51.30	62.77	62.77	45.97	65.21	47.80	57.10	55.78	53.53	49.36	48.59	52.10	7.48
MW	50.89	58.36	66.91	62.70	52.28	37.15	53.88	56.35	59.40	58.18	58.35	56.34	55.74	6.55
GL	41.39	54.70	64.46	63.69	52.75	54.95	48.26	55.41	58.40	57.91	50.77	50.76	53.36	6.02
OUSE														
EP	55.29	59.33	71.45	62.64	55.13	65.09	44.46	61.88	64.80	60.89	58.85	57.54	59.28	6.43
EN	59.95	54.13	72.14	64.92	56.86	65.40	46.87	58.06	63.86	69.39	55.63	51.88	60.06	7.26
BU	58.27	57.18	65.49	61.46	50.03	60.50	48.35	60.83	58.11	55.23	48.44	48.03	55.99	5.95
PP	41.44	55.57	57.78	61.34	58.59	55.83	43.72	51.50	52.78	52.39	47.98	45.03	51.99	6.31
MW	56.50	57.32	68.55	64.32	51.13	58.08	50.99	54.41	56.69	55.58	55.44	54.75	56.98	5.00
GL	53.33	58.63	61.38	69.49	53.32	61.15	43.73	55.81	56.77	54.08	51.39	50.81	55.82	6.48

A three-way ANOVA was performed on the data in Table IV.4.1, the results of which are summarised in Table IV.4.2.

TABLE IV.4.2

ANALYSIS OF VARIANCE OF PERCENTAGE DRY MATTER DATA

(* significant at 5%, ** significant at 1%)

	d.f.	SS	MS	F
Clone (A)	5	665.271	133.054	10.462 **
Site (B)	1	13.950	1.097	n.s.
AB	5	119.810	23.962	n.s.
Time (C)	11	3836.105	348.737	27.421 **
AC	55	1220.093	22.184	1.744 *
BC	11	545.445	49.586	3.899 **
ABC (error)	55	699.510	12.718	

The analysis of variance indicates that there is a significant effect due to clones, time, and the time x site and time x clone interactions.

Further tables showing the incidence of each interaction effect on percentage dry matter were also derived. From these, the behaviour of percentage dry matter of clones at different sites, and of the six clones over time were plotted in Figures IV.4.2 and IV.4.3 respectively. These graphs show that there is no significant difference between the two sites in respect of dry matter, but there are significant differences in percentage dry matter with time for the six clones studied. The observation that there is a significant effect due to clones is also evident again in this analysis.

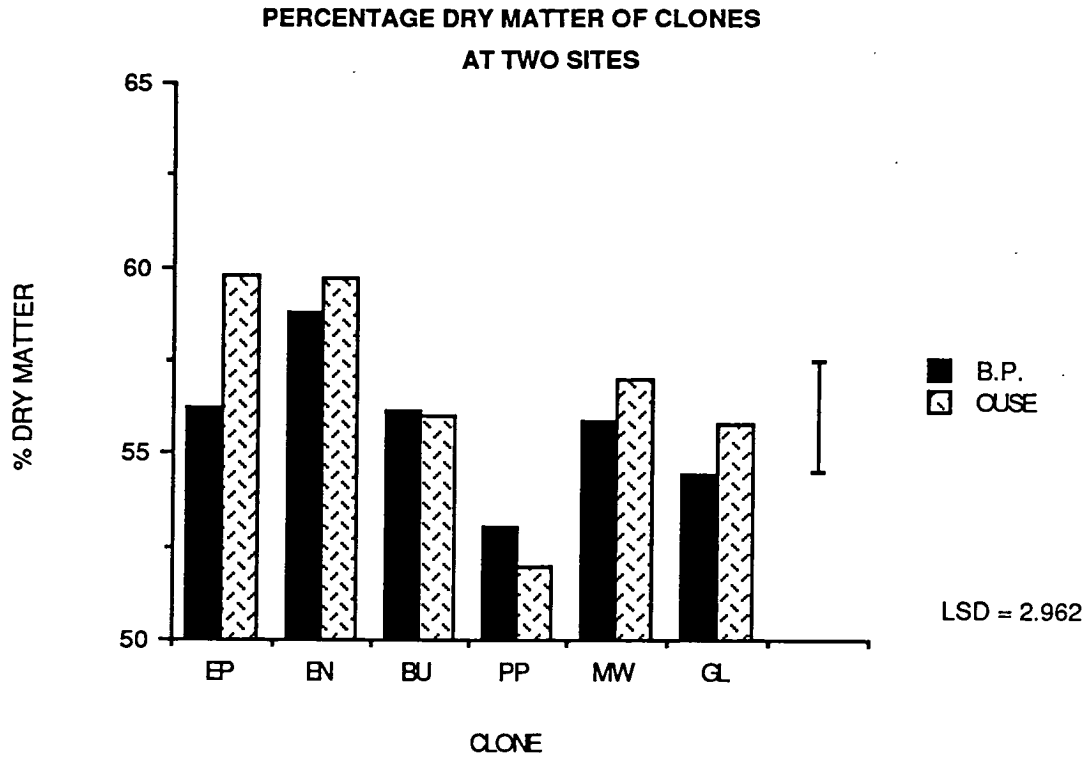

PERCENTAGE DRY MATTER OF CLONES
AT TWO SITES
% DRY MATTER
65
60
55
50
EP
EN
BU
PP
MW
GL
CLONE
B.P.
OUSE
LSD = 2.962

FIGURE IV.4.2

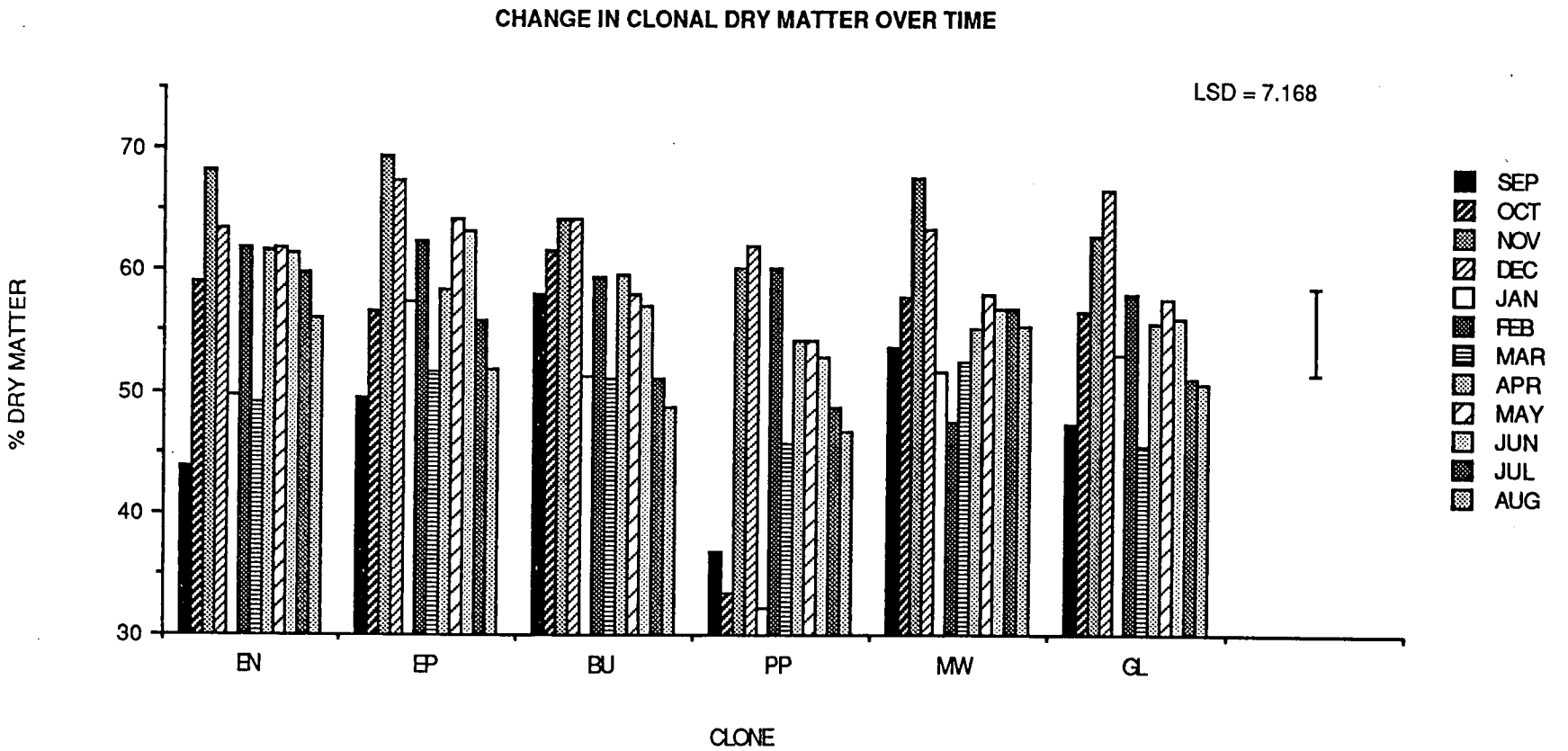

CHANGE IN CLONAL DRY MATTER OVER TIME
LSD = 7.168
% DRY MATTER
70
60
50
40
30
EN
EP
BU
PP
MW
GL
CLONE
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG

FIGURE IV.4.3

4.2 INFRA-RED PHOTOGRAPHIC GROWTH RECORD

A series of infra-red photographs were taken at the Ouse and Bushy Park sites in order to follow the increase in percentage canopy cover for each of the clones. This graphic method was then correlated with growth measurements.

4.2a MATERIALS AND METHODS

Plant Material and Equipment

At both the Bushy Park and Ouse, a block of plants containing all six clones was used as the subject for the infra-red photographs. In each plot of 10 plants, markers were driven in to the ground to show the position in which the photographic frame was to be placed at each successive sample time. The frame itself was 71 x 71 cm from September '87 to January '88 inclusive. From Febuary '88 a new quadrat, 75 x 75 cm was used.

A Canon AE-1 camera was used in conjunction with an Arrow R-60, 55 mm red filter. The film used as Kodak HIE High Speed Infra-red 2481, 36 exposure. In general, a film speed of 1000 was required, with film speed ASA 50 and aperture f/11. To obtain an impression of percentage canopy cover in the selected quadrats, the camera was positioned as near to vertical as possible above the frame.

Photographs were taken for a period of 12 months, at monthly intervals, from September 1987 through to August 1988, inclusive.

Film Development and Processing

The film was handled in total darkness. Kodak D-19 developer was used for maximum contrast; six minutes at 20°C, running water rinse for 30 seconds, followed by fixing in Kodak Rapid Fixer for 2 to 4 minutes. Finally the film was washed in running water for 20 to 30 minutes. The photos were printed using an automated print processor.

Analysis of Photos

A HiPad digitizer was used to analyse the raw data. This was linked to a cross-hair hand piece in conjunction with an Apple IIc computer. The program Bioquant II was used to give an estimate of

percentage canopy cover from the outline of plant material on each photograph.

Each photo was placed on the pad and the cross-hair hand piece was used to trace out the shape of the area of plant material. The cross-hair and digitizer pad were calibrated such that the area of the frame (either 71 x 71 cm or 75 x 75 cm) was 100%.

4.2b RESULTS AND DISCUSSION

The percentage canopy cover figures as computed by the Bioquant II program are tabulated for the Ouse and Bushy Park sites in Table IV.4.3. Figures IV.4.4 and IV.4.5 show the increase in percentage canopy cover at Bushy Park and Ouse, respectively, for the six clones.

The increase in percentage cover established by each clone (at both sites), are shown in the six parts of Figure IV.4.6. Figures IV.4.7 and IV.4.8 show typical photographs from the beginning and the end of the trial for the Mount Wellington (Figure IV.4.7) and Elephant Pass (Figure IV.4.8) clones.

TABLE IV.4.3
PERCENTAGE CANOPY COVER INCREASE WITH TIME

BUSHY PARK

DATE	GL	PP	EP	MW	BU	EN
02/09/87	29.378	24.715	23.712	31.870	27.172	28.036
01/10/88	39.858	31.361	37.197	38.018	32.655	50.000
03/11/87	60.322	43.369	76.136	63.005	69.794	71.647
06/01/88	70.112	55.951	86.839	76.318	77.971	93.343
10/02/88	80.599	73.057	87.036	81.836	91.375	96.563
22/04/88	83.813	83.120	90.174	97.328	100.000	100.000
04/05/88	90.962	85.990	94.440	98.042	100.000	100.000
09/06/88	96.098	88.560	99.170	100.000	100.000	100.000
02/07/88	98.100	92.122	100.000	100.000	100.000	100.000
29/07/88	100.000	99.650	100.000	100.000	100.000	100.000

OUSE

DATE	GL	PP	EP	MW	BU	EN
02/09/87	19.339	10.000	19.480	37.165	13.281	11.401
01/10/87	31.780	16.284	20.098	54.495	23.475	14.594
03/11/87	43.989	32.749	27.165	58.997	35.712	47.413
06/01/88	64.808	55.660	59.598	64.950	98.191	82.306
10/02/88	90.491	76.088	91.977	70.142	100.000	80.145
22/04/88	93.413	90.754	93.198	84.544	100.000	93.198
04/05/88	95.755	96.352	97.677	84.966	100.000	100.000
09/06/88	98.560	96.380	98.570	97.180	100.000	100.000
02/07/88	99.140	96.580	99.110	97.900	100.000	100.000
29/07/88	100.000	97.998	99.255	98.950	100.000	100.000

Over the 332 days of the recorded growth, most clones reached 100% canopy cover. Those that did not were Paradise Plains, Elephant Pass and Mount Wellington at the Ouse site; though, even these covered some 97% of the available ground when the trial was terminated.

The clone that reached total canopy cover the earliest was

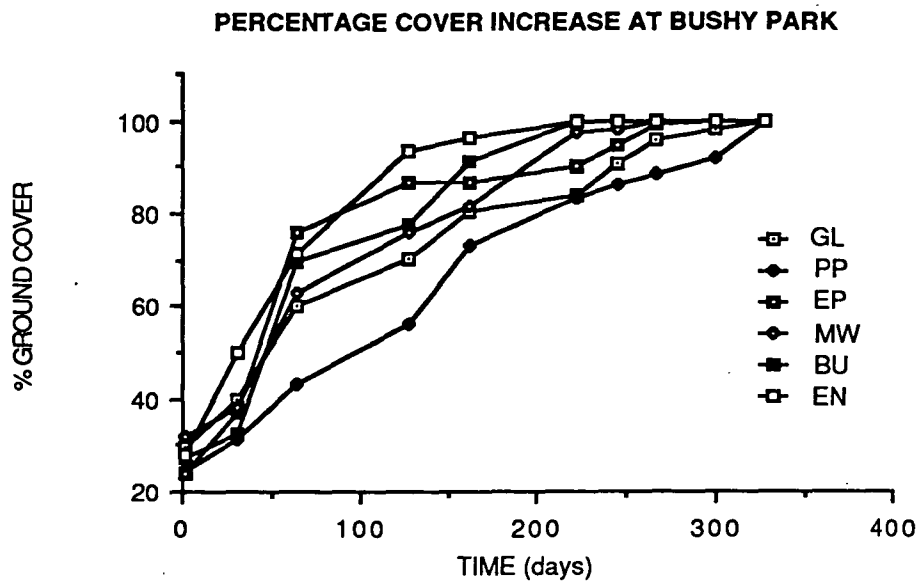


FIGURE IV.4.4

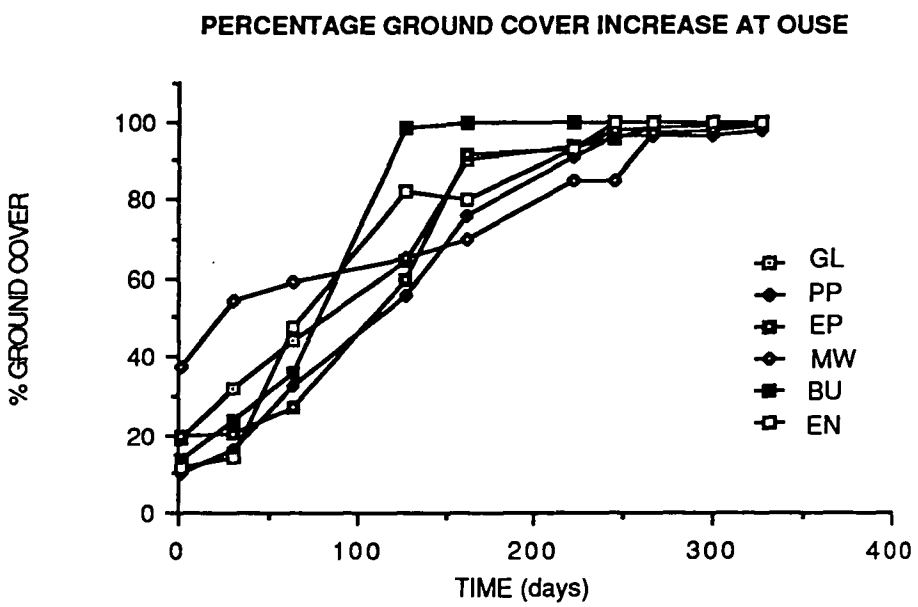
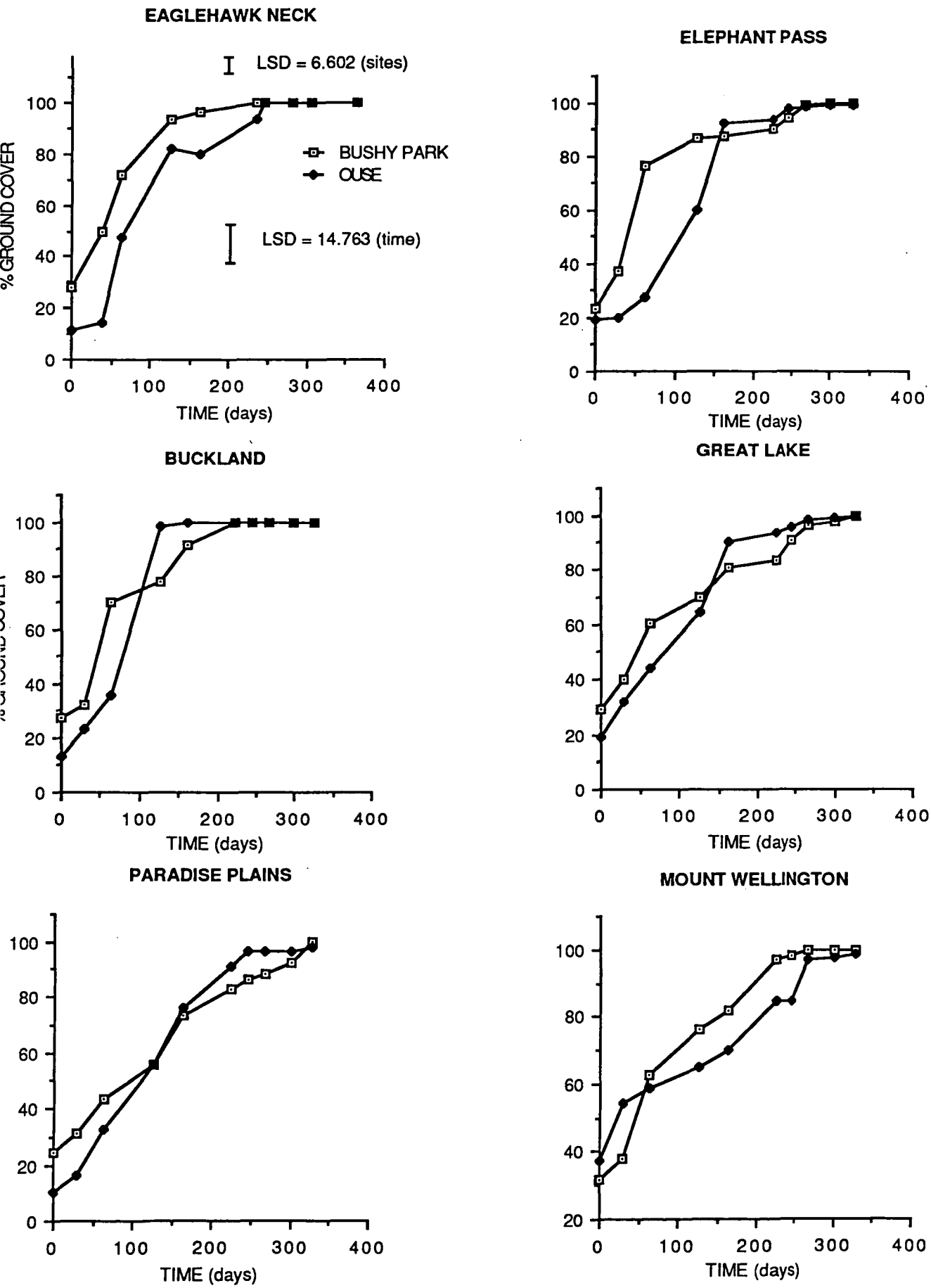


FIGURE IV.4.5

FIGURE IV.4.6

INCREASE IN PERCENTAGE COVER FOR SIX CLONES AT OUSE AND BUSHY PARK

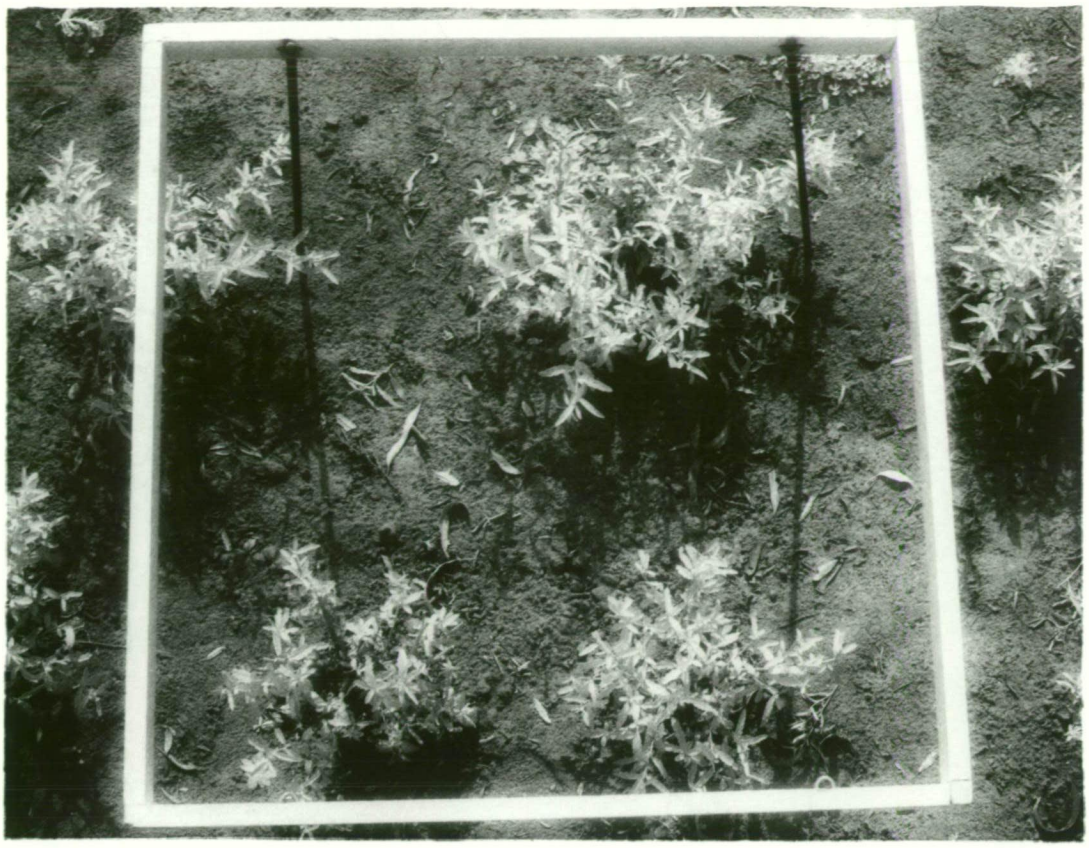


September '87 Bushy Park MW

February '88

FIGURE IV.4.7

INFRA-RED PHOTOGRAPHS TAKEN AT TWO TIMES (BUSHY PARK)



September '87 Ouse EP

February '88

FIGURE IV.4.8

INFRA-RED PHOTOGRAPHS TAKEN AT TWO TIMES (OUSE)

Buckland, growing at the Ouse site, in 162 days. At the Bushy Park site, Buckland and Eaglehawk Neck took 223 days to achieve total ground cover. This rate of increase corresponds with the observation that, at Ouse, Buckland was the most vigorous type, whereas Eaglehawk Neck was the most vigorous at Bushy Park, (see IV.3.3c).

At the Bushy Park site, the infra-red growth monitoring followed the removal of three quarters of the plants' terminal height, in order to reveal the effect of harvest. Even with this pruning regimen, the total ground area was shaded by leaves within 200 days, at the 0.5 x 0.5 m triangular spacing used.

A comparison between relative growth rates and percentage canopy cover increase can be undertaken only between September '87 and February '88 since these are the times when there was an overlap between the canopy cover trial and the growth trial.

It has been shown that 'growth' can best be monitored by observing the height in these plants, (see IV.4.1). In the case of the Ouse site, for instance, the sharpest increases in height occur between November and December. At this time the increase in canopy cover is also very rapid. Naturally, once the growth has established nearly 100% canopy cover, the rate of cover increase slows down, but height increase is unrestricted. (Figure IV.2.1)

The same relationship exists for stem diameter, (see Figure IV.2.2), but is even more graphic for width, at Ouse. Here it can be seen that for the Eaglehawk Neck, Elephant Pass and Buckland clones the increase in width was greatest between November and December, as shown in Figure V.2.3. This is reflected in the percentage canopy cover increases with the steepest slopes at that time. The potential for further increases in width are limited due to plant spacing and habit.

4.3 PRUNING TRIAL

Another important crop management consideration is the pruning regime. The aim is to find a height to which to prune the plants, such that the maximum yield is obtained.

4.3a COMPARISON OF VIGOUR RESPONSE TO THREE PRUNING REGIMES

A pruning trial was initiated at Bushy Park in August '87, in order to determine the recovery response of a selected clone to pruning. For this experiment, the Elephant Pass type was used.

Three levels of pruning were used, namely the removal of 1/4, 1/2 and 3/4 of terminal height. The number of plants under each pruning regimen was:

Treatment	Pruning Height	No. of Plants
A	1/4	34
B	1/2	56
C	3/4	40

All of the plant heights were measured at monthly intervals for six months from August '87 to February '88 inclusive.

Table IV.4.4 shows the incremental height increase of plants in each pruning treatment over the 246 days of the trial.

TABLE IV.4.4

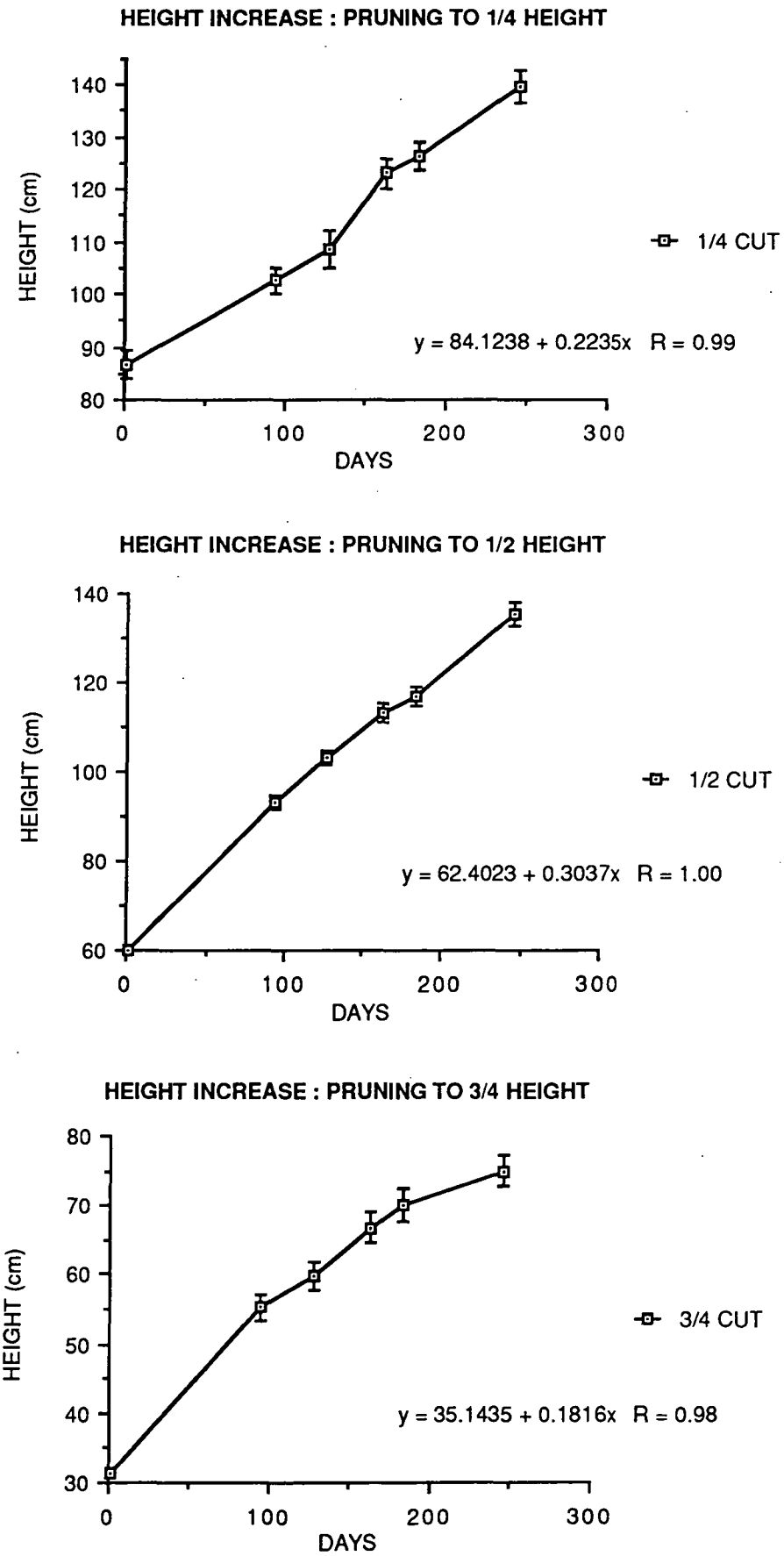
AVERAGE INCREMENTAL HEIGHT INCREASE AND GROWTH RATE FOR THREE PRUNING REGIMENS

Trt.	Initial Height (cm)	Final Height (cm)	Increment (cm)	Mean Growth Rate (cm/day)
A	86.7	145.1	58.4	0.1916
B	60.1	142.8	82.7	0.2709
C	31.4	90.1	58.7	0.1925

Treatment B plants had the greatest response to pruning in terms of growth achieved per day, and their final height was similar

FIGURE IV.4.9

HEIGHT INCREASE IN RESPONSE TO THREE PRUNING REGIMES



to that of plants in treatment A. Thus, the large growth increment resulted from the relatively smaller plant. This may be attributed to greater vigor when unproductive old wood is removed.

Figure IV.4.9 shows the way in which height increased over time for the three treatments. The plants pruned to half their initial height (Treatment B) reached terminal heights equal to that of plants which had only one quarter of their height removed (Treatment A). On examination of the Treatment A and Treatment B plants it was found that they produced new growth only on the end of cut laterals, and below about 0.75 m, the stems were woody and bore only mature or senescent leaves.

Figure IV.4.10 shows the relative growth rates for the three treatments. These were calculated by assigning the beginning of the trial as day 0, and determining the height increase relative to this initial value.

The trend is for all pruning regimes to result in similar growth rates, until the onset of the new seasons' growth period towards the end of the trial. Treatment B plants responded before the others with a large increase in relative growth rate. However, this was followed by a sharp decrease during the final month. The plants which had most foliage removed (Treatment C), responded with a sharp increase in relative growth rate at the final measurement. At this point, plants under the other two treatments are tending towards negative relative growth.

It is perhaps of commercial interest that the rapid increase in relative growth rate, associated with severe pruning, as in treatment C, occurs at the time when oil yield has reached its maximum.

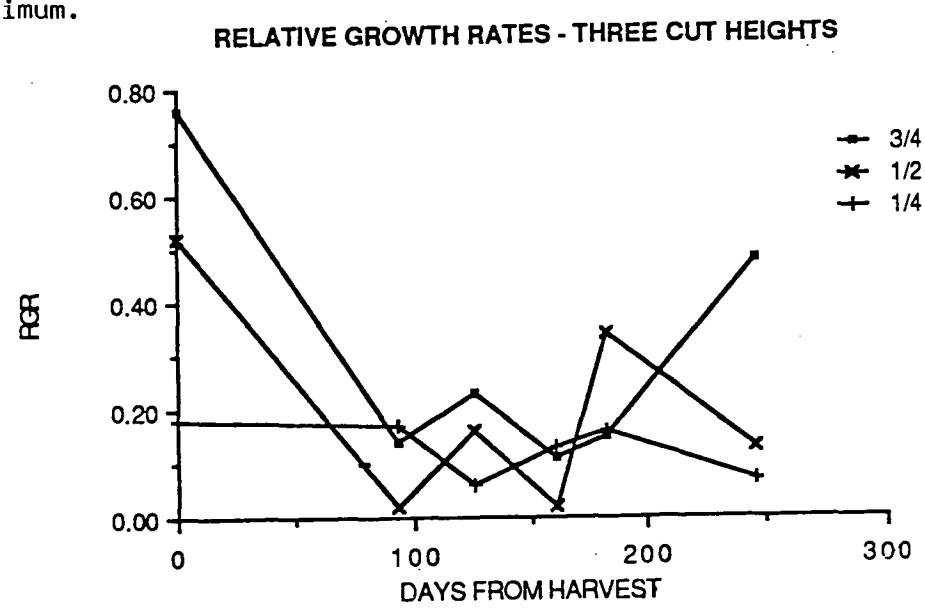


FIGURE IV.4.10

4.3b COMPARISON OF OIL YIELD FROM PLANTS UNDER DIFFERENT PRUNING REGIMES

Essential oil yield from woody stem material which bear only mature or senescent leaves is minimal, and the hard, woody stalks are difficult to cut. It has been shown that oil yield is greatest from young current seasons' growth (see Seasonal Variation Trials), and harvest should encourage the production of as much of this type of material as possible. Thus, the yield from plants maintained at a low level, say 30 cm, would be proportionally more per kilogram of harvested material than plants pruned to 90 cm.

Samples which were taken for dry matter and percentage oil yield determinations reflected the expected trends. Thus:

TREATMENT	% DRY MATTER	% OIL YIELD (d.m.b.)
A	72.27	0.037
B	57.26	0.069
C	54.59	0.189

The plants that had the greatest amount of material removed at the time of pruning, that is to 3/4 of their height, produced a multitude of new shoots from the previous old wood. Growth was not limited to terminal extensions, but encompassed an abundance of new shoots which originated from points low on the main stem. Thus, from the point of view of attempting to maximise oil yield, this treatment would provide the more rewarding results. It limits the production of low yielding woody material which is uneconomical to distill, while maximising the amount of young oil-rich shoots available.

5. SEASONAL VARIATION TRIALS

Once the pruning method has been established, the time of year at which it is implemented will effect both yield and organoleptic quality of the oil.

5.1 FERN TREE SEASONAL VARIATION TRIAL

The seasonal variation trial at Fern Tree was initiated in January 1987. Five plants were selected at the site and laterals were chosen and marked for collection of samples over the period of the trial.

Samples were collected at the beginning of each month. Juvenile and mature growth was taken from marked laterals. The juvenile growth was regarded as that from the apex to six nodes below the apex. Sufficient leaf material was collected for small scale solvent extraction; that is, some 2.5 to 3 g. The samples were processed and analysed by gc as detailed in Materials and Methods.

From the gc trace the response obtained from the internal standard was used to determine the yield as detailed in Materials and Methods.

In addition, chromatograms were analysed for component variation. The components were:

1. α-pinene
2. β-pinene
3. 1,8-cineole
4. unknown
5. germacrene-D
6. bicyclogermacrene
7. spathulenol
8. γ-eudesmol
9. β-eudesmol
10. internal standard
11. unknown
12. unknown

The percentage composition of each component was transformed to g of the component so that all measurements could be quantitatively compared. Plots were constructed of μg component (x) versus time.

5.1a VARIATION IN ESSENTIAL OIL YIELD

The graphs in Figures IV.5.1 and IV.5.2 show the percentage oil yield (on a dry matter basis, d.m.b) over time for each of the five replicate plants. Figure IV.5.1 shows the trend for current growth and Figure IV.5.2 for the mature growth. These two graphs show that material from all five plants follows a similar oil yield trend.

Figure IV.5.3 contains the means of the yields of the five plants for both types of material collected. Table IV.5.1 presents a list of these figures.

TABLE IV.5.1
AVERAGE MONTHLY ESSENTIAL OIL YIELDS (d.m.b.)
BY SOLVENT EXTRACTION (g)
FERN TREE SEASONAL VARIATION TRIAL

MONTH	JUVENILE	MATURE
JAN '87	0.9073	0.5655
FEB	1.3043	0.8437
MAR	0.9891	0.6501
APR	0.6421	0.5897
MAY	0.9955	0.6684
JUN	0.9282	0.7641
JUL	1.2917	0.9387
AUG	0.7271	0.4960
SEP	0.8233	0.5355
OCT	0.8836	0.5691
NOV	1.2762	0.6343
DEC	0.5440	1.3007
JAN '88	1.2166	0.7119
FEB	0.7488	0.2899

The current growth has three distinct peaks occurring in February, July and November-December. The summer peak may be a continuous one from November through to February, or it may be dependent on rainfall conditions. The Spring-Summer season in 1987-88 was uncharacteristically dry, as shown by the rainfall figures in Table IV.5.2. For example, the rainfall in January 1987 was 42 mm above the average, whereas in January 1988 it was 35 mm

below the monthly average. This is reflected to some extent in the dry matter results. In January 1987 % dry matter was 42.31% and January 1988 it was 52.61%.

TABLE IV.5.2

TEMPERATURE AND RAINFALL DATA FOR PERIOD JAN '87 TO FEB '88

MONTH	MIN°C	MAX°C	TOTAL RAINFALL (mm)	AVERAGE RAINFALL/MTH (mm)
JAN '87	15.1	21.3	118.0	76
FEB	15.8	21.9	56.0	78
MAR	12.1	18.6	96.8	102
APR	11.7	19.3	31.2	84
MAY	9.6	15.4	114.0	93
JUN	7.1	12.3	65.4	79
JUL	7.9	12.0	79.0	112
AUG	8.4	14.5	82.0	112
SEP	10.3	17.0	72.0	102
OCT	10.6	17.5	99.0	105
NOV	14.3	19.6	110.0	129
DEC	12.8	19.9	118.0	108
JAN '88	14.0	24.3	41.0	76
FEB	11.9	21.0	28.0	78

Note: These readings were taken at the Hobart station since this was the closest recording position to the Fern Tree site.

Table IV.5.3 gives the dry matter results for the 14 months of the trial. The influence of the available moisture on essential oil content in Olearia may well be a contributing factor to the seasonal oil yield peaks observed in this trial. As a result of these observations, a glasshouse trial was initiated to attempt to clarify the relationship between available moisture and percentage oil yield in Olearia. See IV.5.1c.

TABLE IV.5.3

MEAN DRY MATTER OF O. PHLOGOPAPPA AT FERN TREE

SEASONAL VARIATION TRIAL

MONTH	MEAN % DRY MATTER
JAN '87	42.31
FEB	45.22
MAR	49.66
APR	58.49
MAY	47.03
JUN	45.78
JUL	46.58
AUG	54.32
SEP	57.18
OCT	48.96
NOV	49.95
DEC	57.22
JAN '88	52.61
FEB	50.02

The mature growth follows a similar trend in oil content, over time, to the current growth, but the only significant increase occurred in February 1987.

Observations show that the current apical growth bears consistently more oil than leaves which have fully matured. This is the reverse to the situation observed in many other essential oil crops e.g. peppermint (Clark R.J. and Menary R.C., 1984).

During one of the periods of lowest yield, that is April, the percentage oil content in both types of leaves is not significantly different. After the summer flush of growth, few new leaves are being produced. Therefore, the plant bears leaves which are mostly physiologically mature. The differences that occur between leaves of different physiological ages, compared to different chronological ages, were investigated in the Seasonal Variation Trial at Bushy Park. See IV.5.2.

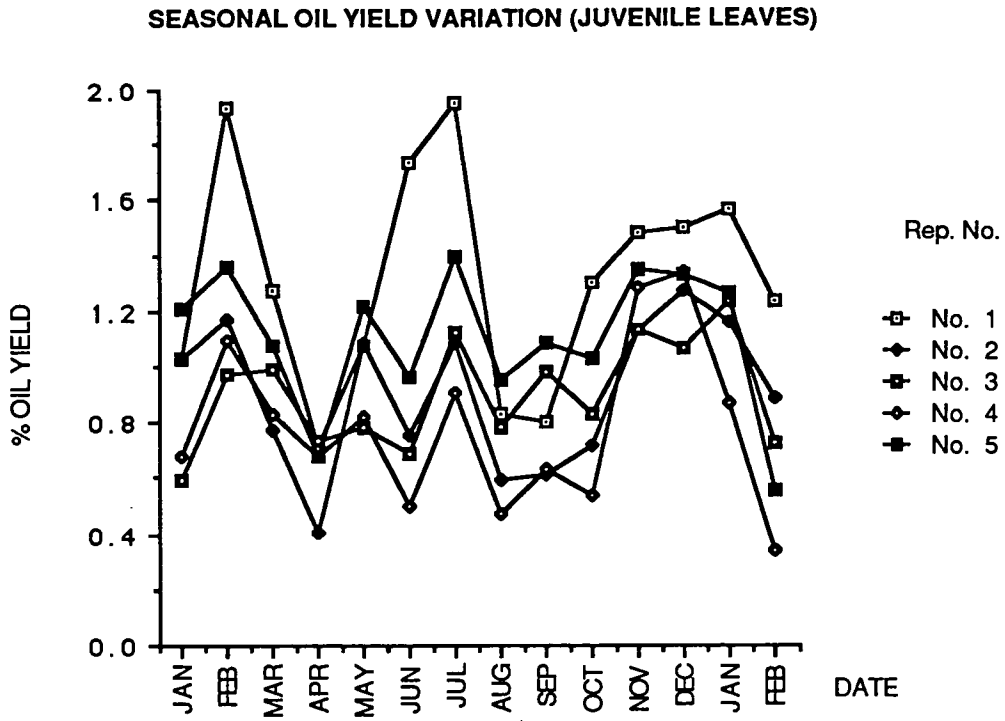


FIGURE IV.5.1

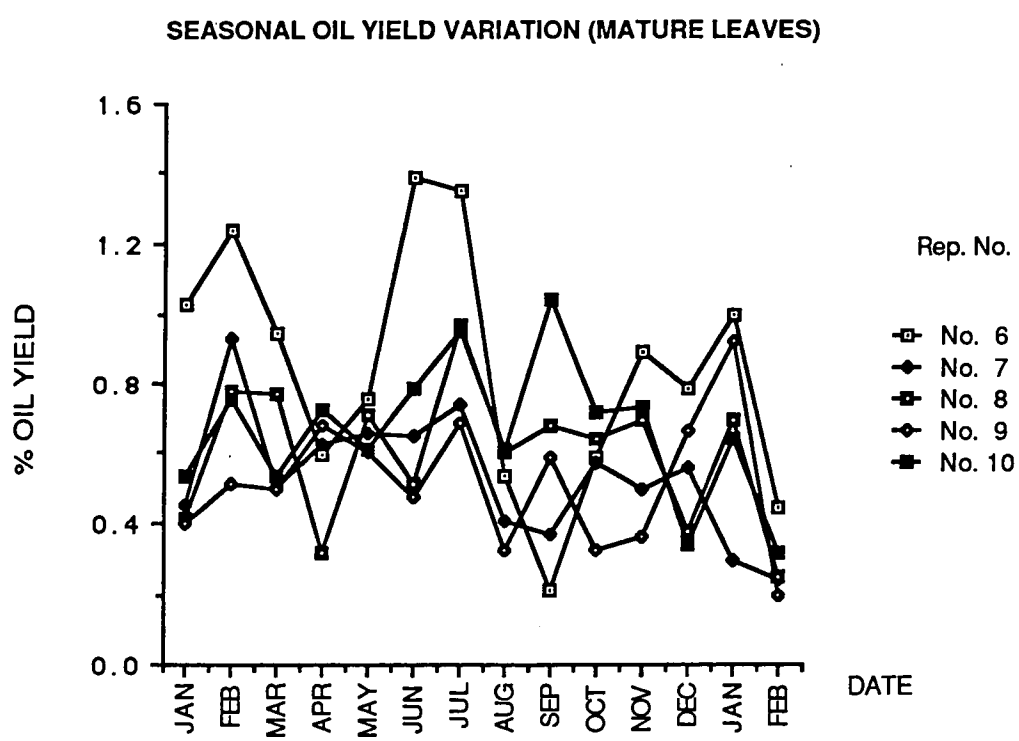


FIGURE IV.5.2

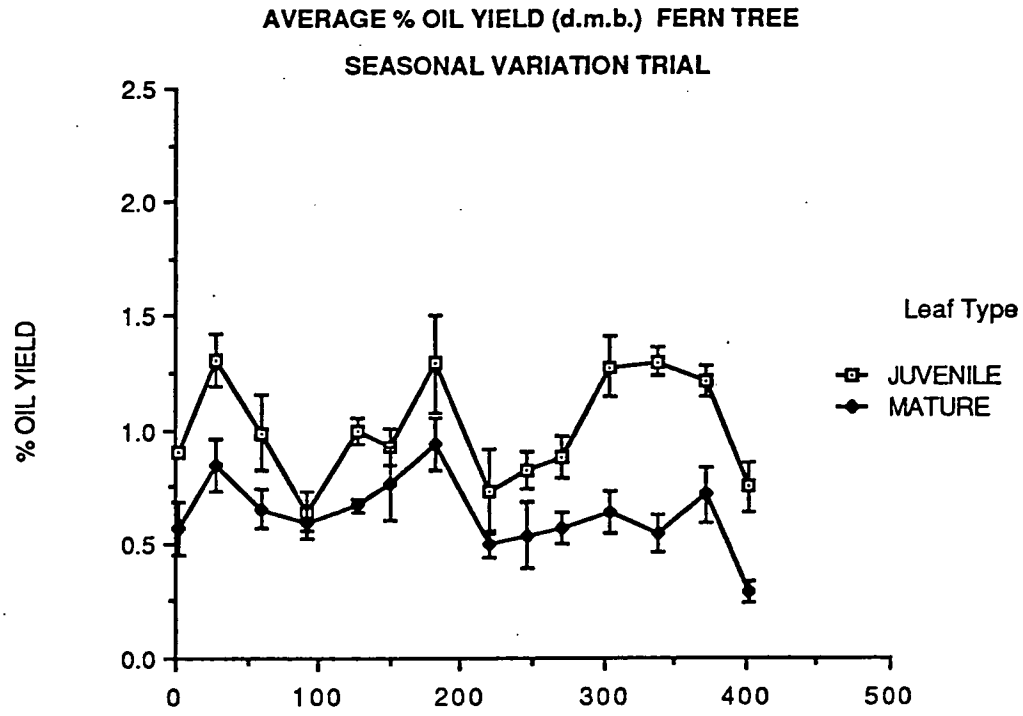

AVERAGE % OIL YIELD (d.m.b.) FERN TREE
SEASONAL VARIATION TRIAL
% OIL YIELD
2.5
2.0
1.5
1.0
0.5
0.0
0
100
200
300
400
500
Leaf Type
JUVENILE
MATURE

FIGURE IV.5.3

5.1b VARIATION IN ESSENTIAL OIL QUALITY

The quality of oil from leaf samples collected during the trial was monitored by selecting 12 major peaks from the gas chromatograms. These were simply labelled 1 to 12. The amount of each of these components present in the oil was determined in µg by including an internal standard during the extraction procedure. The selected peaks are shown in the representative chromatogram in Figure IV.5.4. Component numbers 1,2 and 3 are monoterpenes, (α-pinene, β-pinene and cineole, respectively). The remainder are sesquiterpenes except for number 10 which is the n-octadecane internal standard.

Each month, from January '87 to February '88, the variation in each of the twelve components was recorded. The results are presented as the twelve parts of Figure IV.5.5, where each individual peaks' seasonal variation is shown.

The following observations were made:

Component 1 (α-pinene)

The younger leaves tend to contain more of this component than older leaves, at all times, with peaks in April, September and January '88. The pattern followed tends to be the same as that for percentage oil yield. From May to November (Days 127 to 305), the difference between the mature and juvenile types is not significant.

Component 2 (β-pinene)

There is a tendancy for the yield of this component to be greater in mature growth in September. Peak yields occur in February '87 and March. The March peak is most distinct and the older material has a similar amount as the juvenile material. In the mature growth component 2 falls away to virtually zero around January and February.

Component 3 (1,8-cineole)

Juvenile and mature yields are inverted in April and May. That is, they are reversed from the norm of current leaves yielding more that mature leaves. Yields peak in February-March and September. The juvenile growth experienced a significant increase in component

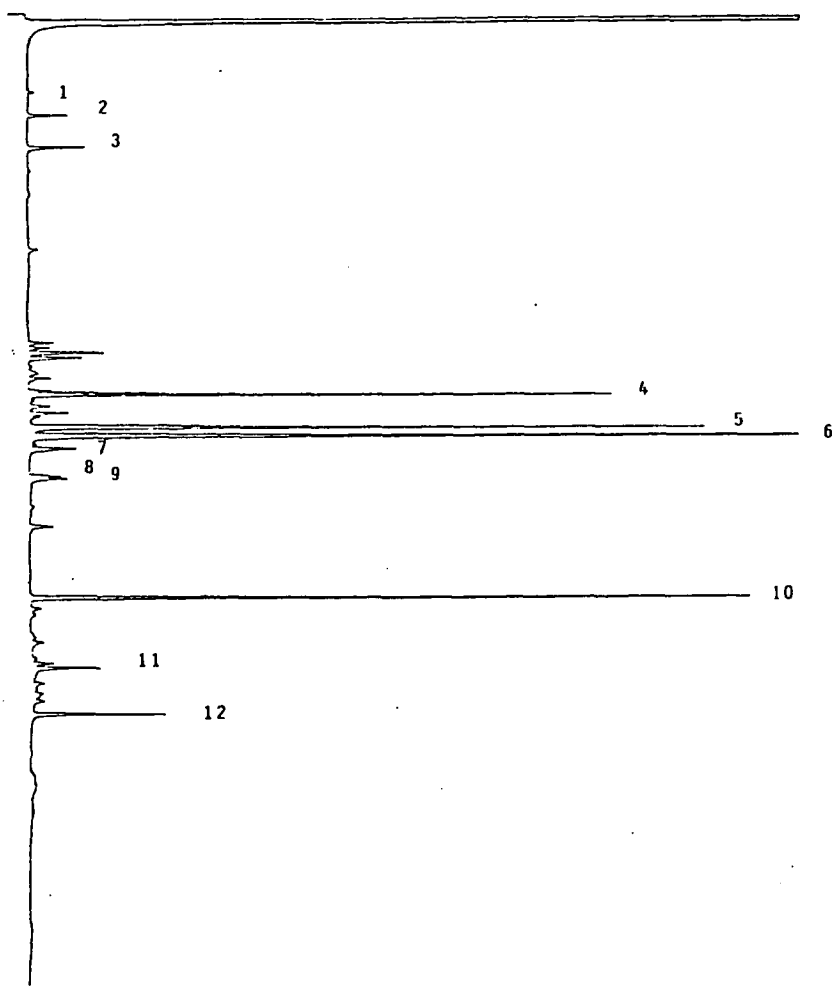


FIGURE IV.5.4

GAS CHROMATOGRAPHY TRACE OF TYPICAL ESSENTIAL OIL FROM THE FERN TREE SEASONAL VARIATION TRIAL

1.	α-pinene	7.	spathulenol
2.	β-pinene	8.	γ-eudesmol
3.	1,8-cineole	9.	β-eudesmol
4.	caryophyllene	10.	internal standard
5.	germacrene-D	11.	unknown
6.	bicyclogermacrene	12.	unknown

3 during the Summer growth season, peaking in January '88.

1,8-cineole has a similar overall trend to α-pinene.

Component 4 (caryophyllene)

This sesquiterpene has a higher concentration in older tissue in April, May and June. Thus, it is dominant in the older leaves in winter. It peaks in February-March, September and December-January.

Component 5 (germacrene-D)

Germacrene-D peaks in February-March, September and December-January. With this component, the differences in yield between mature and juvenile growth are not significant throughout the year.

Component 6 (bicyclogermacene)

Mature growth yields greater amounts of bicyclogermacrene than juvenile growth, only in April, but the yields are not statistically significantly different. Peaks occur in February, September and December. Juvenile material contained significantly more of component 6 in November-December and January-February '88.

Component 7 (spathulenol)

This component has peaks in yield in April, June and September for mature leaves. At other times the levels are low in both leaf types.

Component 8 (γ-eudesmol)

Yield is highest in older material in September, exceeding that in young leaves. Peaks are observed in April, September and December. With this component a significant difference between yield in older leaves and yield in younger leaves occurs only in September.

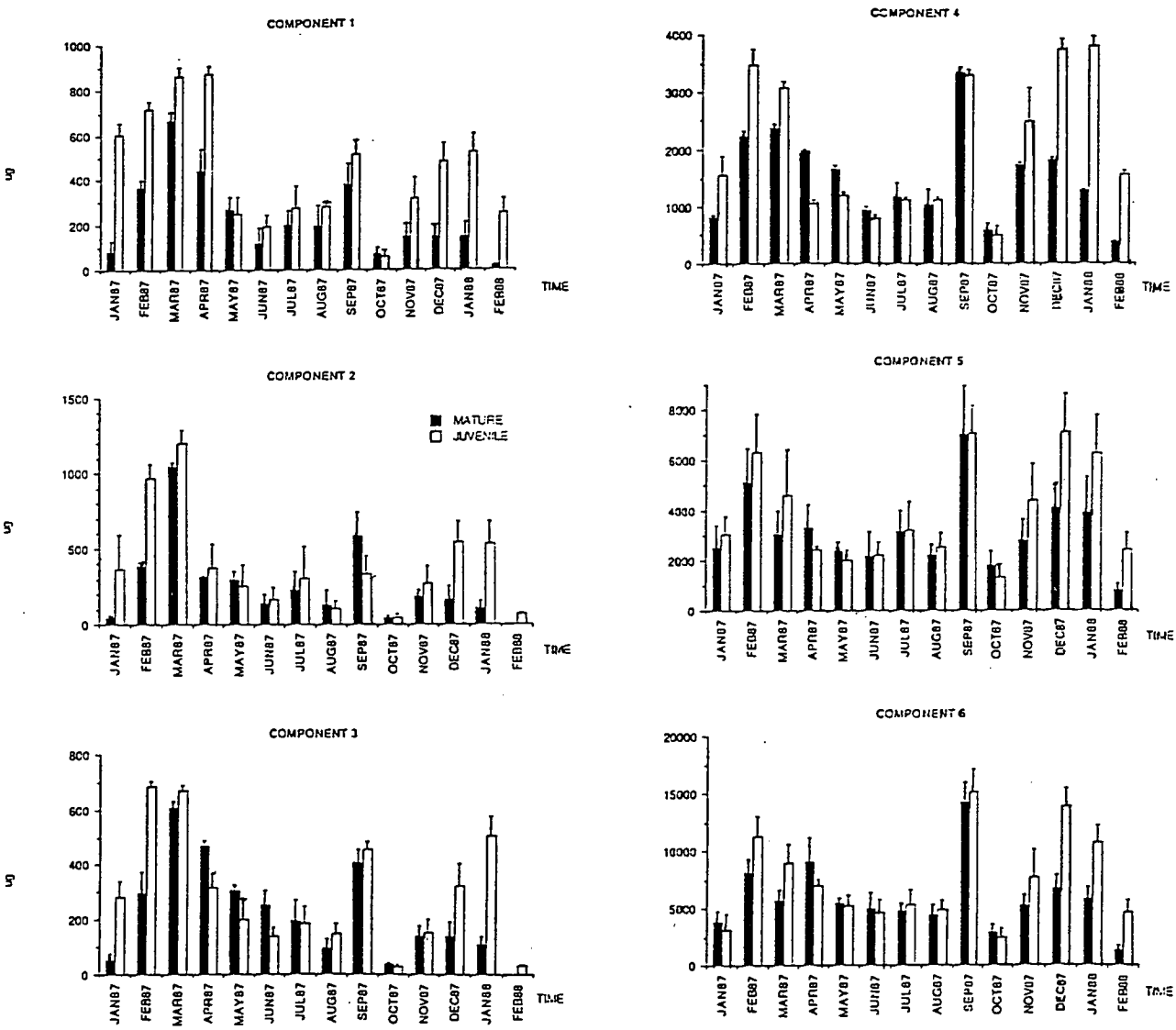


FIGURE IV.5.5a

VARIATION OF COMPONENTS 1 - 6 IN THE ESSENTIAL OILS FROM THE PRELIMINARY SEASONAL VARIATION TRIAL AT FERN TREE

(Quantities are µg/µl injection volume)

Component 9 (β-eudesmol)

Component 9 shows yields which do not follow the total percentage oil yield pattern. The level is constant throughout the year except in November when the yield of oil in current growth increases dramatically. Note, however, that this peak is preceeded by a smaller one, in mature material, in September.

Component 10 (n-octadecane internal standard)

The amount added was 1 ml up to and including April. Thereafter, 0.5 ml was used in the preparation of all solvent extraction samples.

Component 11 (unknown)

The only significant peaks occurred in the young material in April, January-February '88, and in June in older leaves, though the overall yield of this component does not vary significantly throughout the year.

Component 12 (unknown)

Yields were significantly different and increasing in November-December. Otherwise the amounts of this constituent remain quite constant. June also showed a large increase in this compound in younger leaves.

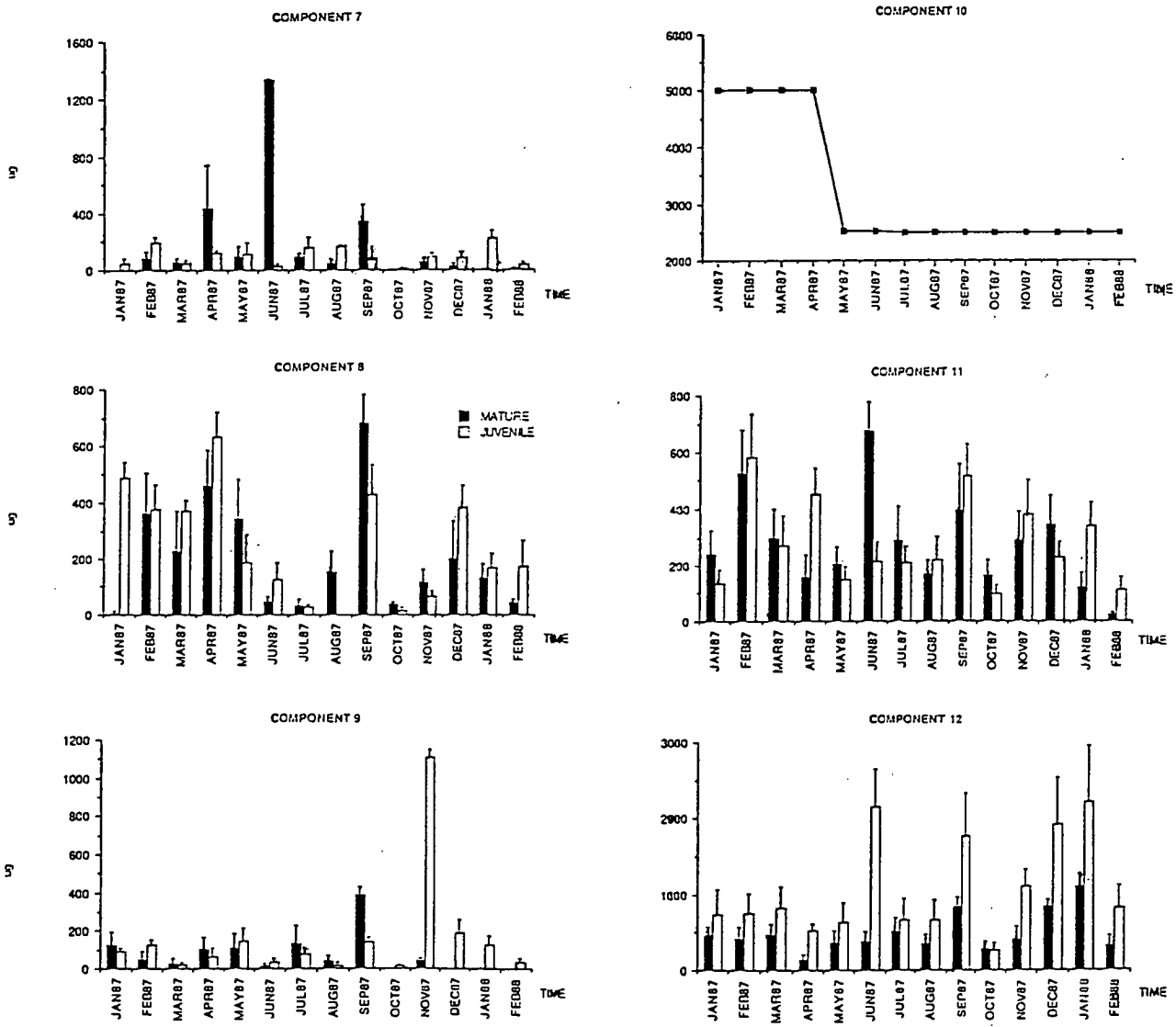


FIGURE IV.5.5b

VARIATION OF COMPONENTS 7 - 12 IN THE ESSENTIAL OILS FROM THE PRELIMINARY SEASONAL VARIATION TRIAL AT FERN TREE

Note: Component 10 is the C_{18} internal standard

(Quantities are µg/µl injection volume)

5.1c MOISTURE STRESS GLASSHOUSE TRIAL

I. EFFECT OF MOISTURE STRESS ON ESSENTIAL OIL YIELD AND DRY MATTER CONTENT IN MW

The moisture stress trial aimed to establish the effect, if any, of a decrease in available moisture on the essential oil production of Olearia. Both the yield and quality of the oil were examined.

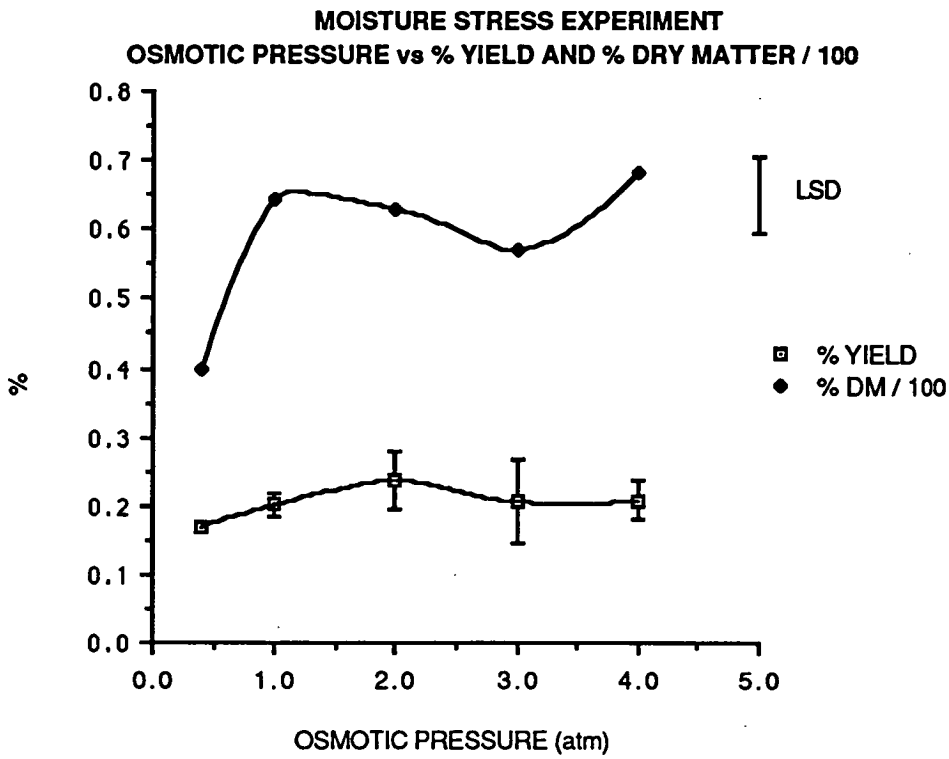
The quality of the oils were assessed at the end of the trial by destructively harvesting leaf and stem material for solvent extraction. The samples were prepared with an internal standard by the method described in Materials and Methods. The percentage composition for seven constituents was determined by gas chromatography and was collated for analysis.

The change in oil yield and percentage dry matter with increasing moisture tension are shown in Figure IV.5.6. The percentage dry matter has been scaled by a factor of 100 to enable it to be displayed with the same vertical axis as oil yield. The nature of the relationship between osmotic pressure and either of percentage oil yield or dry matter may not be linear, since regression analysis shows that correlation coefficient values of 0.64 and 0.48 are obtained for oil yield and dry matter, respectively.

Oil yield has a tendency to increase with increasing moisture tension, however, the changes are not significant, as indicated by the standard errors of the data.

The pattern of fluctuation of percentage oil yield is closely followed by the fluctuation in percentage dry matter. See Figure IV.5.6. Thus, the addition of PEG results in a decrease in water held by the plant. The percentage dry matter increases as PEG is added to the growing medium, and maintains a plateau throughout the range of osmotic pressures applied.

As a consequence of the above results, it may be postulated that the occurrence of water stress in the range of 0.4 to 4.0 atm, has little, if any, effect on the quantity of extractable oil produced by Olearia plants. However, the levels of oil content do


MOISTURE STRESS EXPERIMENT
OSMOTIC PRESSURE vs % YIELD AND % DRY MATTER / 100
%
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.0
1.0
2.0
3.0
4.0
5.0
OSMOTIC PRESSURE (atm)
LSD
% YIELD
% DM / 100

change from one season to the next in plants growing under normal environmental conditions, as seen in the Fern Tree Seasonal Variation Trial. Thus, it may be that the trial was not of sufficiently long duration for significant changes to occur in percentage oil content, or that the range of osmotic pressures in the solution cultures were inadequate to demonstrate these effects.

Using Figure III.2 in Materials and Methods, which shows the relationship between the percentage moisture content and osmotic potential of soils from Ouse, Bushy Park and Fern Tree, an attempt can be made to extrapolate the observed results to the field condition. It is evident that even at 5 atm soil moisture tension, the soils at Ouse, Bushy Park and Fern Tree are at 8 %, 9 % and 22 % soil moisture respectively. Evidence from the glasshouse trial suggests that even at these moisture levels, there should be no significant effect on oil yield. At 1.0 atm, the respective soil moisture contents are 11.9%, 13.6% and 31.6 %. It could be expected that when these levels are reached an increase in percentage dry matter will occur, but will not increase further with decreasing water content.

Work by Allen S.G and Nakayama F.S (1988), has brought to light evidence that in some plants, there is no difference in net photosynthesis between plants that were well watered and those that were grown under drought treatment. If this is true in this case, changes in oil yield would not be expected to directly result from less assimilate. Rather, they occur as a consequence of altered partitioning of available resources.

The number of oil glands per unit area was not found to be directly related to percentage oil yield (See Figure IV.1.4, WILD POPULATIONS). So percentage yield is not altered by the number of glands present. In most cases then, it seems that a change in gland size is responsible for the change in yield. Lower yield could be attributable to small glands, or unfilled glands. The number of glands may well be pre-determined during leaf initiation, being genetically controlled.

FIGURE IV.5.7

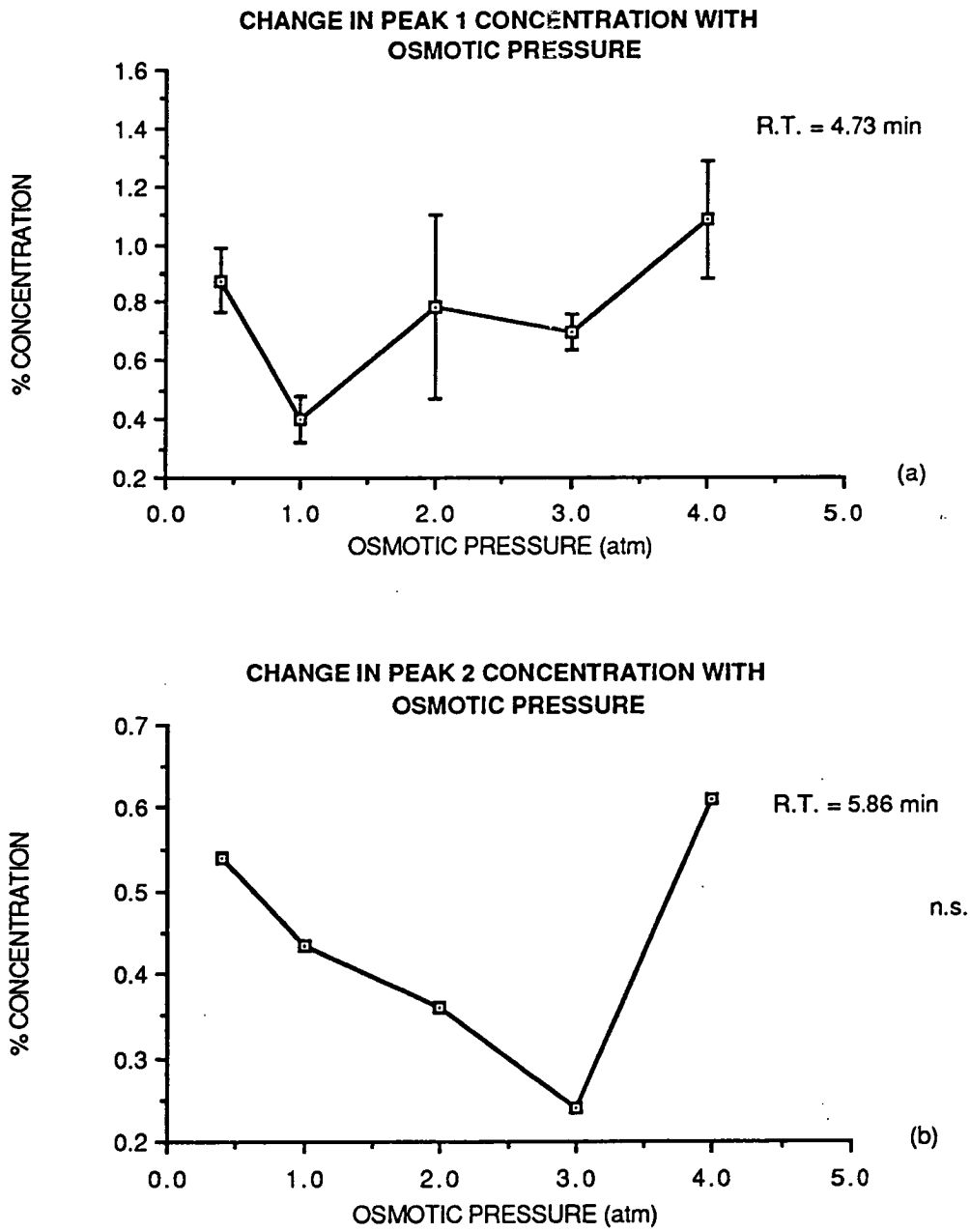
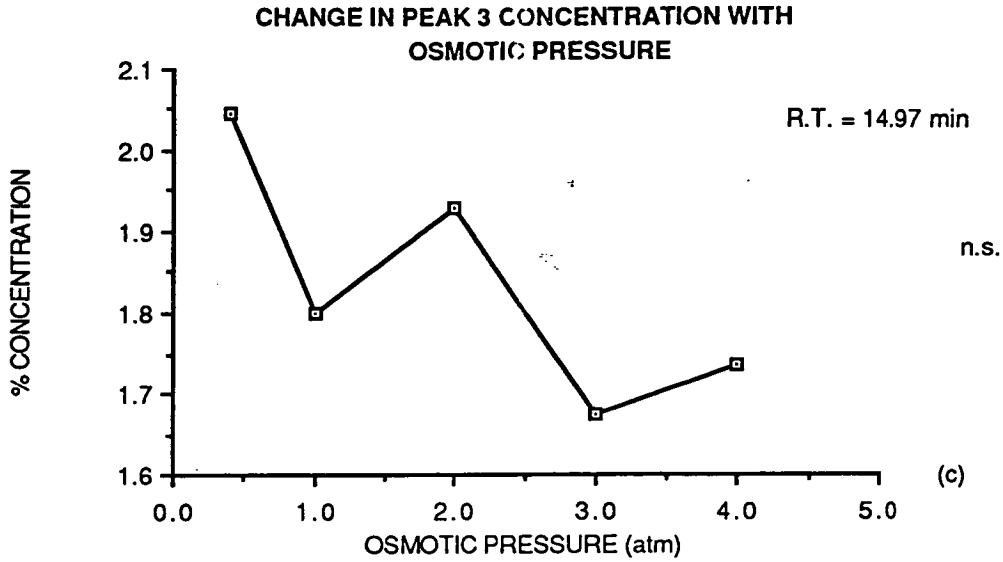
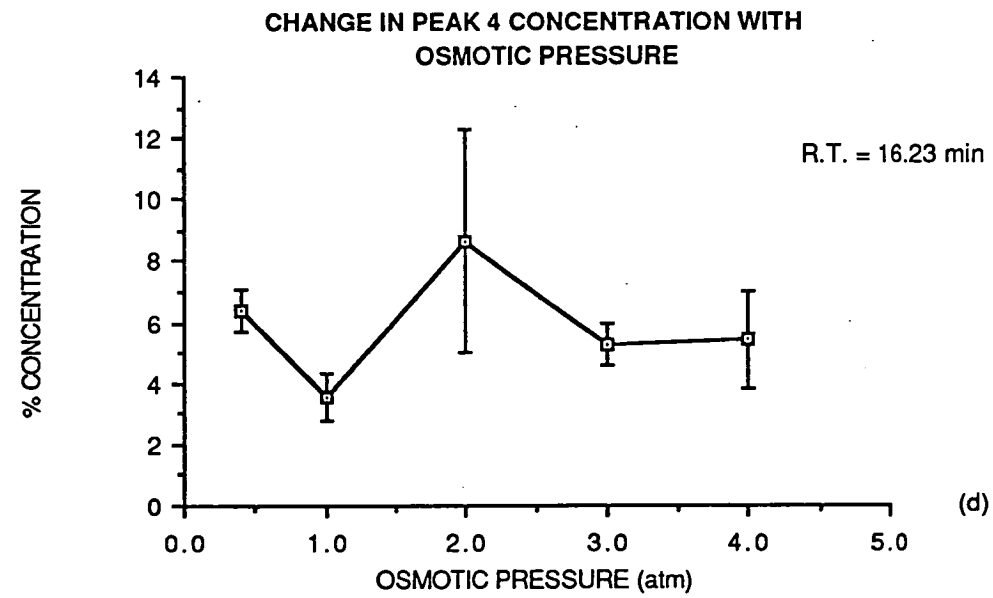

CHANGE IN PEAK 1 CONCENTRATION WITH OSMOTIC PRESSURE
R.T. = 4.73 min
% CONCENTRATION
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1.0
2.0
3.0
4.0
5.0
OSMOTIC PRESSURE (atm)
(a)
CHANGE IN PEAK 2 CONCENTRATION WITH OSMOTIC PRESSURE
R.T. = 5.86 min
n.s.
% CONCENTRATION
0.7
0.6
0.5
0.4
0.3
0.2
0.0
1.0
2.0
3.0
4.0
5.0
OSMOTIC PRESSURE (atm)
(b)

FIGURE IV.5.7 cont.





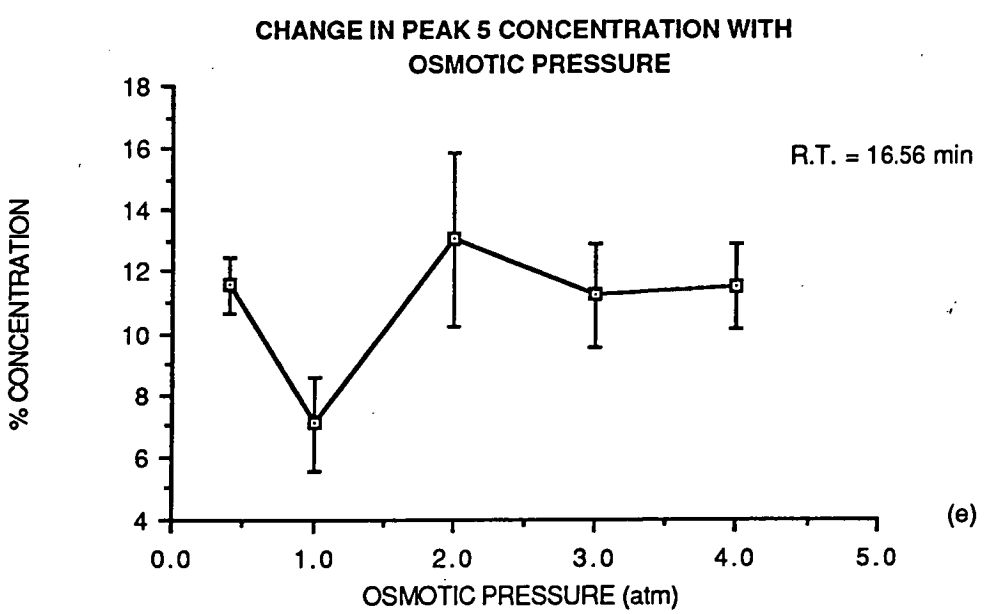
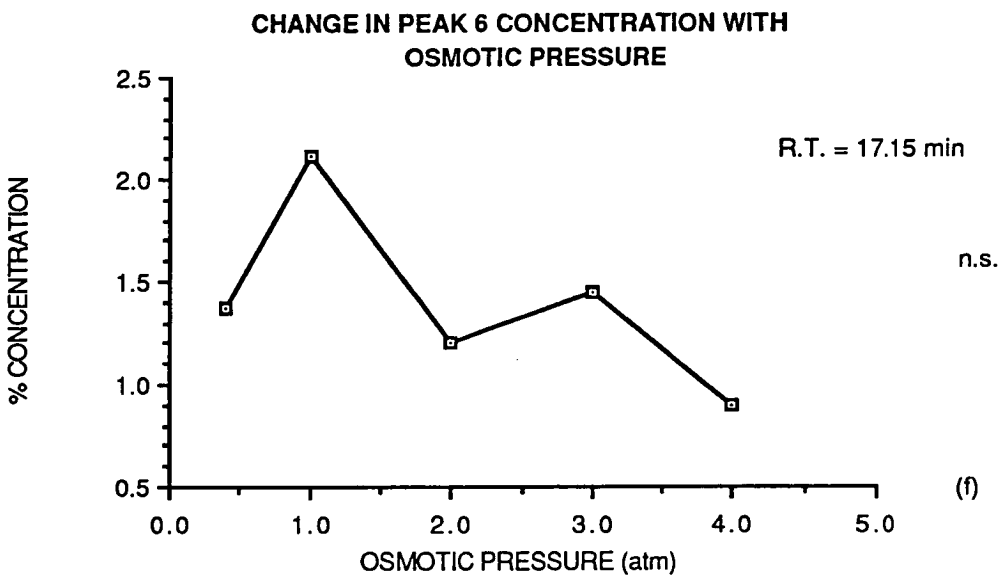
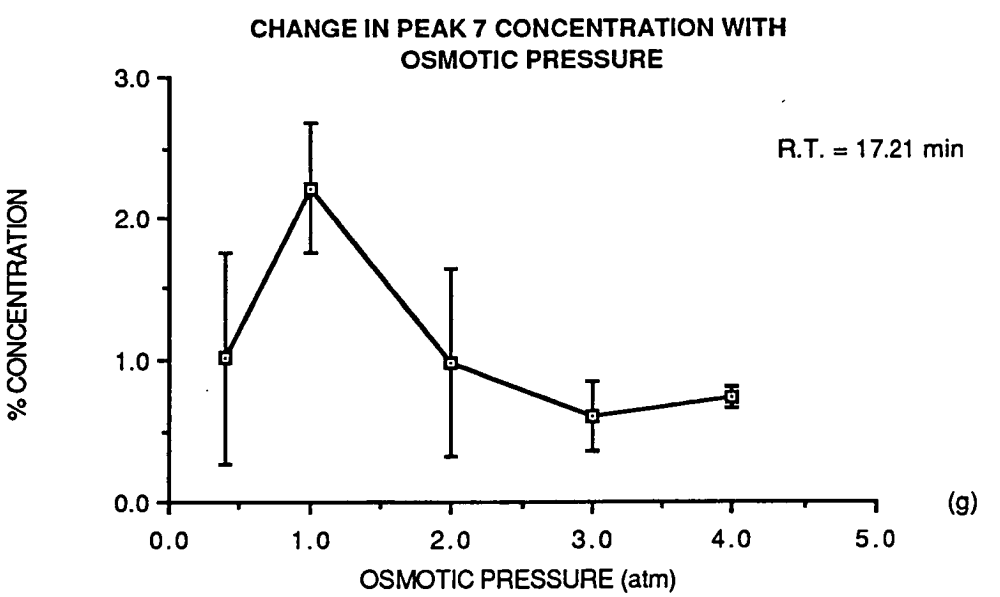


FIGURE IV.5.7 cont.





II. OIL COMPONENT VARIATION WITH MOISTURE TENSION

The composition of the oils from the various moisture stress treatments was surveyed by choosing seven components of the oil and monitoring them by gas chromatography. Plots showing the effect of change in osmotic pressure on each of the seven components are given in Figure IV.5.7 (a,b,c,d,e,f and g). The retention times of the seven peaks were:

Peak 1	4.73	β-pinene
Peak 2	5.86	1,8-cineole
Peak 3	14.97	caryophyllene
Peak 4	16.23	germacrene-D
Peak 5	16.56	bicyclogermacrene
Peak 6	17.15	liguloxide
Peak 7	17.21	kessane

The percentage composition data is given in Appendix D.

Of the seven components studied, three did not alter significantly with change in osmotic pressure. These were peaks 2, 3 and 6.

The remaining peaks responded to changing osmotic pressure as follows. The peak 7 level increased significantly at an osmotic pressure of 1.0 atm. In contrast, peak 1, 4 and 5 concentrations fell significantly at 1.0 atm. In addition, peak 1 levels again increased to a maximum of 1.1% when the greatest moisture stress was imposed.

In the range of osmotic pressures studied, the composition of the oil is altered the most at 1.0 atm. Some changes in oil quality are therefore to be expected under mild moisture stress conditions. Nevertheless, the interaction of the osmotic and seasonal effects, particularily at time of harvest, should be borne in mind, especially when consistent oil quality is desirable.

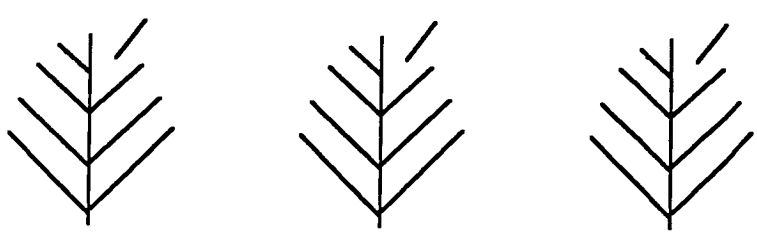
5.2 SEASONAL VARIATION TRIAL CONDUCTED AT BUSHY PARK

This experiment aimed to monitor the variation of oil quantity and quality in:

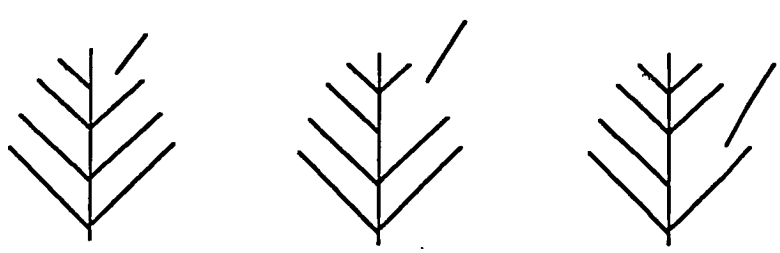
1. leaves of the same physiological age with time, that is, chronological changes, (referred to as Type 1 leaves), and
2. leaves of increasing physiological age with time, that is, physiological changes, (referred to as Type 2 leaves).

Material suitable for the first experiment was obtained by collecting the third fully expanded leaves from subject plants. Leaves were designated for the second experiment as follows. The third fully expanded leaf on new laterals was marked with paint. Enough leaves were marked to provide adequate material for collection for the duration of the trial. As they aged, the marked leaves were collected. Diagrammatically, the sampling scheme for the two trials is shown below:

1.



2.



The trial was begun in December '87 with samples being taken at monthly intervals over a period of six months. Towards the end of that time the leaves that had been labelled for collection as

samples for the second experiment, had reached senescence.

Actual collection dates and their identifying labels are listed below:

04/12/87	DEC
06/01/88	JAN
10/02/88	FEB
02/03/88	MAR
04/04/88	APR
04/05/88	MAY

In selecting clones for this experiment the attributes of optimum leaf size and yield of essential oil were considered paramount to enable ease of handling. It was necessary to use clones with large leaves so that enough material could be obtained to give a reasonable yield of extract. At each collection, three replicates of 2 to 3 grams each were taken. The essential oils were derived by solvent extraction with hexane, and they were analysed by gc with an n-octadecane internal standard included in the samples, as detailed in Materials and Methods.

The types chosen were Elephant Pass, Buckland and Paradise Plains.

These were located as follows within the established growth trial plot :

BLOCK

I	C	B	D	E	A	F	
II	E	C	F	A	B	D	where B is PP
III	F	E	D	B	C	A	C is EP
IV	D	A	C	E	F	B	E is BU and

each plot contains ten plants.

5.2a PERCENTAGE OIL YIELD

Figures IV.5.8, IV.5.9 and IV.5.10 show the way in which the percentage oil yield varied in Buckland, Paradise Plains and Elephant Pass over the six month period, respectively. The results for the increasing chronological age and physiological age experiments are shown together for each of the clones. In general, with increasing physiological age the yield of oil decreased. Some estimated 60% of the marked leaves had senesced by March, which brought with it a decrease in oil-bearing tissue per gram of leaves collected. By May there were no marked leaves remaining on the plants, and the trial was terminated.

Over the time that the trial was run, an increase in chronological age resulted in a decrease in yield after the summer growth season.

Leaves from the Buckland clone, of both increasing chronological and physiological age, decline in oil yield to February, increasing in March, and decreasing again to May. The yield of oil is insignificant in Type 2 leaves in May since senescence had begun. Type 1 leaves still contain some 0.15% oil at that point, (Figure IV.5.8).

Leaves from the Paradise Plains clone of increasing chronological age (same physiological age) have a constant oil yield up to April, at which time there was a decline, followed by a significant increase in May. The increase in May again corresponds to a decline in yield from the Type 2 leaves, (Figure IV.5.9).

The Elephant Pass clone shows an increase in oil yield to over 2% from Type 1 leaves in January, followed by a decrease to 0.6% in May. Type 1 leaves always have higher yields than Type 2, (Figure IV.5.10). Leaves of increasing physiological age in both Elephant Pass and Paradise Plains follow the same pattern of percentage oil yield. There is an increase from December to January, followed by a significant decrease in February. March seems to be another peak period, after which senescence begins and the oil yield falls to zero in May. In contrast, the Buckland clone shows no second peak oil yield in leaves of increasing physiological age. There is a steady decline from December to May.

By comparison, the leaves of increasing chronological age (same

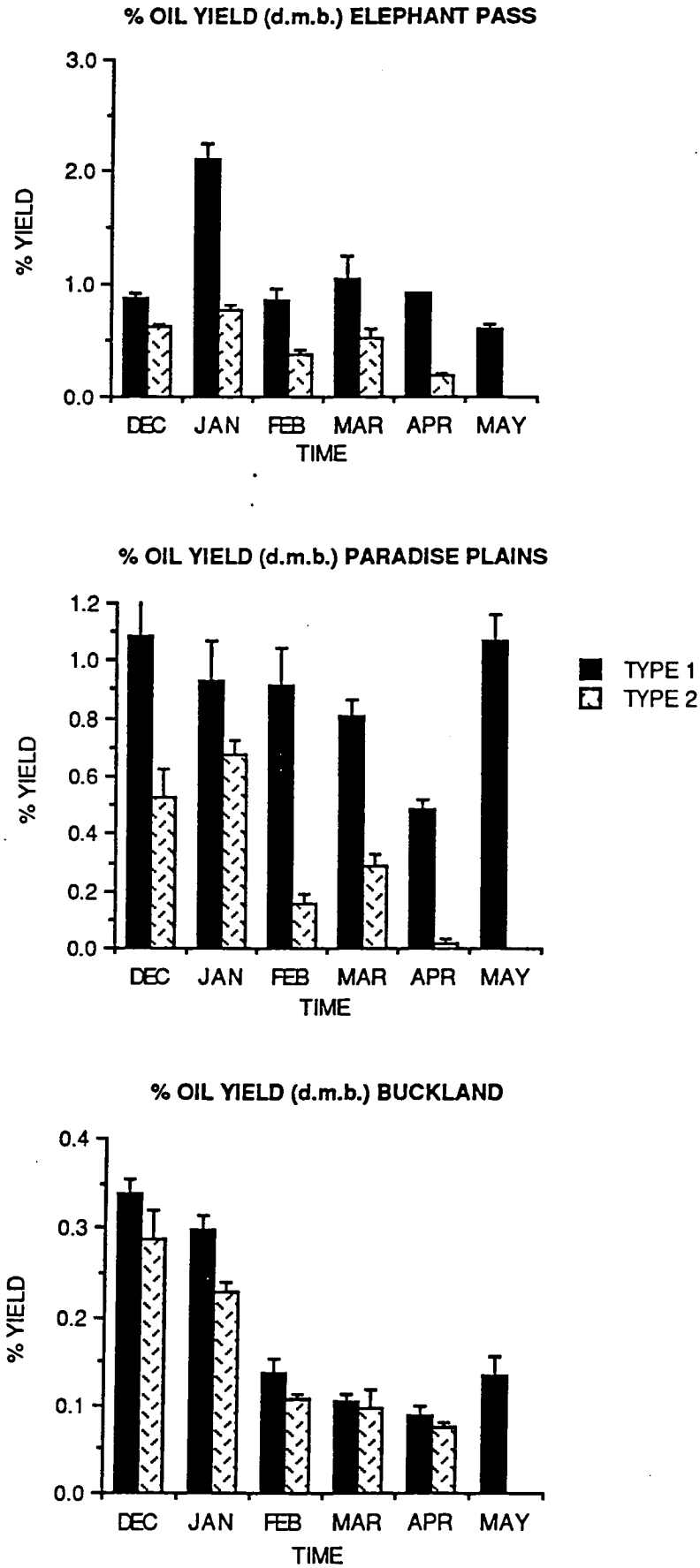

% OIL YIELD (d.m.b.) ELEPHANT PASS
3.0
2.0
1.0
0.0
% YIELD
DEC
JAN
FEB
MAR
APR
MAY
TIME
% OIL YIELD (d.m.b.) PARADISE PLAINS
1.2
1.0
0.8
0.6
0.4
0.2
0.0
% YIELD
DEC
JAN
FEB
MAR
APR
MAY
TIME
TYPE 1
TYPE 2
% OIL YIELD (d.m.b.) BUCKLAND
0.4
0.3
0.2
0.1
0.0
% YIELD
DEC
JAN
FEB
MAR
APR
MAY
TIME

FIGURE IV.5.8

physiological age), in Buckland and Paradise Plains show an increase in oil yield going into May. This is not evident with the Elephant Pass type, which has a single peak yield in January, followed by a steady decline.

By way of summary, there are a few generalisations which can be made. Firstly, the oil yield is consistently greater in physiologically younger leaves than from mature ones. This is in contrast with observations from some other essential oil plants, where oil yield is higher in mature leaves.

Secondly, the maximum oil yields occur between December and February. This response may be somewhat controlled by the prevailing weather conditions.

Lastly, different clones have somewhat individual responses to seasonal changes. The increase in physiological age brings either a steady decrease in percentage oil content, or a more complex reaction, where the decrease is gradual but interupted by an intermediate peak.

5.2b VARIATION IN ESSENTIAL OIL COMPOSITION

In an effort to monitor the changes that occurred in essential oil quality, four components of the Buckland and Elephant Pass extracts, and six components of the Paradise Plains extract were monitored using the gas chromatographic determination of concentrations. Absolute quantities were determined (in mg), using an internal standard during extraction. Comparisons were made between the same component at different times and in different clones.

The results of these determinations are shown in Figures IV.5.9. IV.5.10 and IV.5.11 from Buckland, Paradise Plains and Elephant Pass, respectively.

BUCKLAND

Refer to Figure IV.5.9.

Component 1 (unknown. retention time 13.59 min)

With increasing physiological age this component decreased steadily to zero (senescence), suggesting it is mobilised out of the leaf as it ages, or is converted to some other component. The same component was virtually stable in the young leaves (of the same physiological age), during the trial.

Component 2 (caryophyllene, retention time 13.64 min)

The trends observed for component 1 are again visible for this constituent. The level of caryophyllene in the type 1 leaves is always higher than in leaves of type 2.

Component 3 (germacrene-D, retention time 14.91 min)

The effect of both increasing chronological and physiological age is to decrease the level of component 3. It may be that this compound is converted into another product, rather than transported from one site of accumulation to another.

Component 4 (bicyclogermacrene, retention time 15.25 min)

Component 4 reflects a similar situation to that with components 1 and 2.

The fact that not all the selected components behave the same

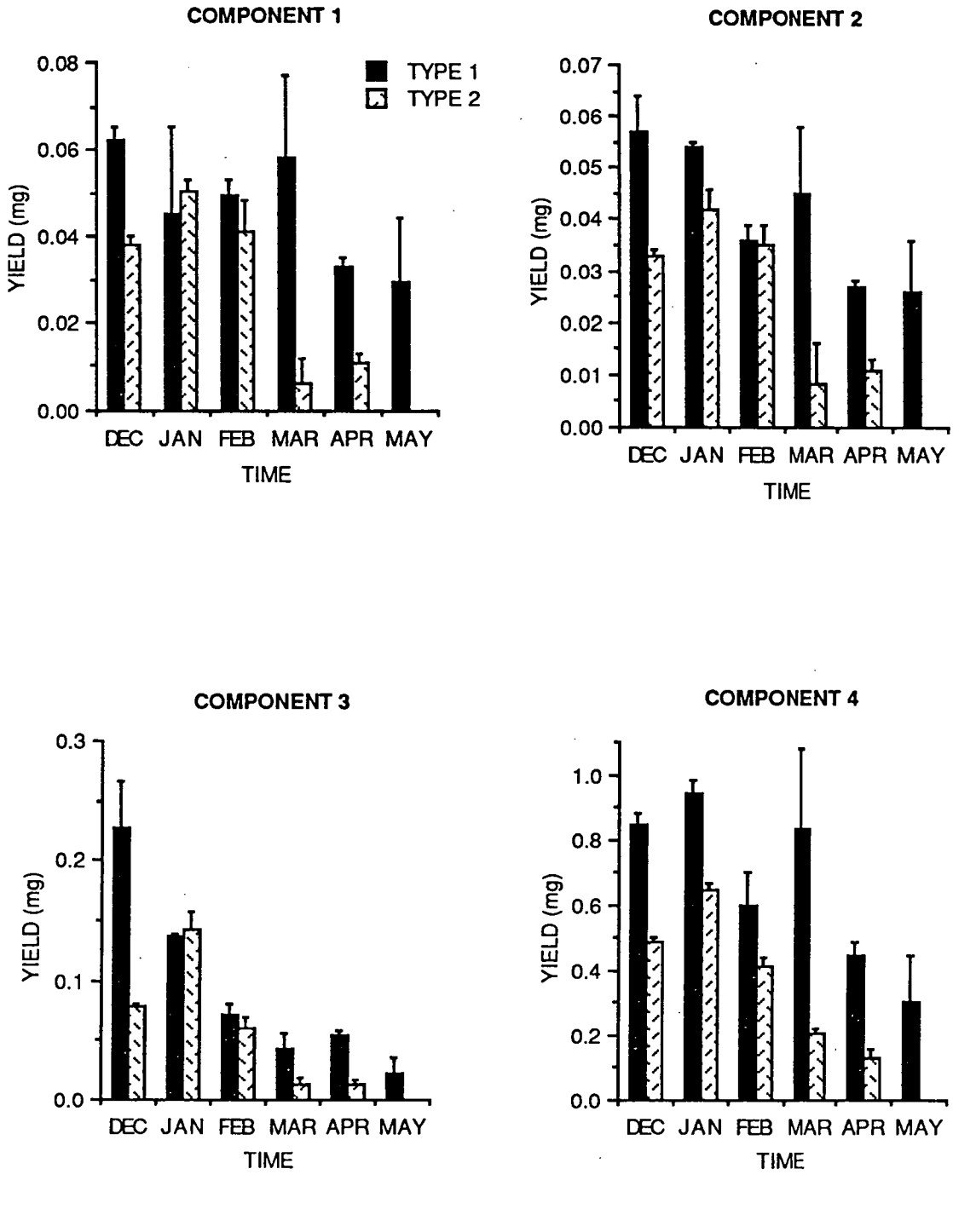


FIGURE IV.5.9

SEASONAL VARIATION OF ESSENTIAL OIL COMPONENTS

BUCKLAND

way over time means that the quality of essential oil is not stable. Its constituents fluctuate in concentration depending on leaf age and time of year.

PARADISE PLAINS

Refer to Figure IV.5.10.

Component 1 (linalool, retention time 4.36 min)

The concentration of Component 1 in Paradise Plains leaves rose and fell over the six month trial. Initially Type 1 fell from December to January, while Type 2 leaves gained in Component 1 concentration, so that both leaf types held the same level. From January, the amount of Component 1 in both types of leaves fell in February, rose in March and fell once more in April. However, Type 2 leaves now contained none of this constituent, and senescence of these leaves had occurred to a large extent. The proportion of Component 1 increased markedly in May to reach a level similar to that observed at the beginning of the trial in December.

Component 2 (bicyclogermacrene, retention time 15.25 min)

The level of bicyclogermacrene is almost constant in the leaves of similar physiological age (type 1). In type 2 leaves, there is an increase from December to January, then a gradual decrease to zero. Thus, the season has little effect on the concentration of bicyclogermacrene in juvenile material.

Component 3 (spathulenol, retention time 16.21 min)

With respect to this constituent, both leaf types followed a similar trend, except that Type 2 always contained significantly less than Type 1. There was a decrease from December to February, an increase in March, after which Type 2 leaves began senescence and the proportion of component 3 decreased to zero in May.

Component 4 (β-eudesmol, retention time 18.07 min)

Component 4 increases from December to May in type 1 leaves. (The April figure may have been spurious, due to sample handling). Type 2 leaves showed an increase from December to January, then a decline, with senescence in April and levels falling to zero thereafter.

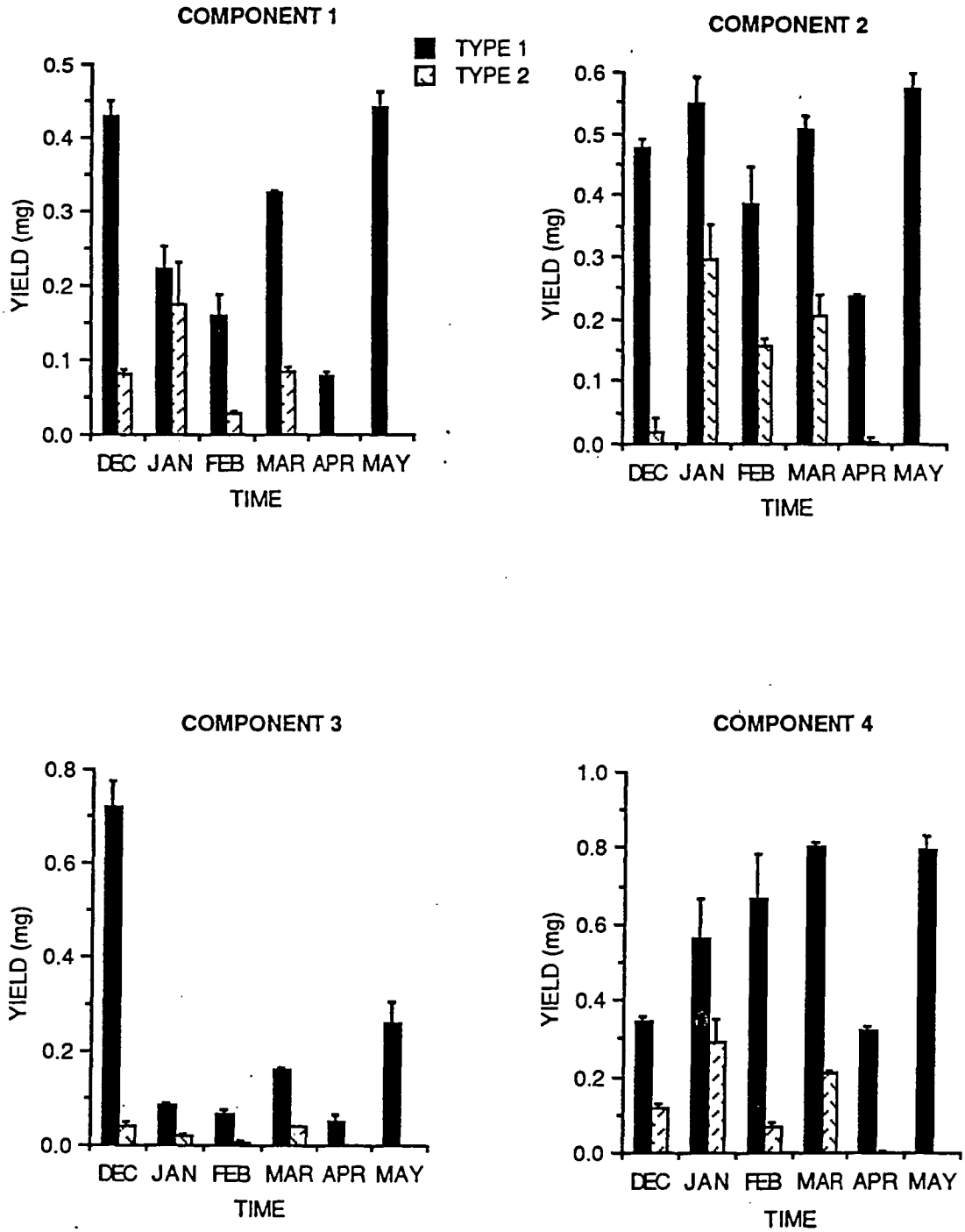


FIGURE IV.5.10

SEASONAL VARIATION OF ESSENTIAL OIL COMPONENTS

PARADISE PLAINS

It may be that all the April figures are low, in which case the pattern of component accumulation or dispersal becomes simple in each oil. There are four different situations represented by the compounds above.

1. Rapid decrease followed by a gradual increase
2. Gradual decrease followed by a gradual increase
3. Gradual increase followed by a steady state
4. Steady state throughout

All type 2 leaves showed a decrease in component level to zero at senescence. This implies that the essential oil is progressively translocated to the younger leaves, or converted into other metabolites.

ELEPHANT PASS

Refer to Figure IV.5.11.

Component 1 (unknown, retention time 13.68 min)

The proportion of Component 1 is always larger in Type 1 leaves than in Type 2 leaves. In December it is approximately equal in both leaf types. It remains constant in Type 1 leaves, with only a slight increase in April-May. This is the time when the Type 2 leaves are senescing and a decrease in component 1 is observed at this time.

Component 2 (germacrene-D, retention time 14.98 min)

Type 1 leaves hold a relatively constant quantity of this component. Type 2 leaves over the same period steadily lose Component 2. It may be that it is recovered from the leaves before they senesce.

Component 3 (bicyclogermacrene, retention time 15.29 min)

As for Component 2, Component 3 is stable in Type 1 leaves, with only a slight decrease in February. Component 3 decreases to zero in May. During December the two leaf types are closest in physiological age. At this time, the level of bicyclogermacrene is the same in both leaf types.

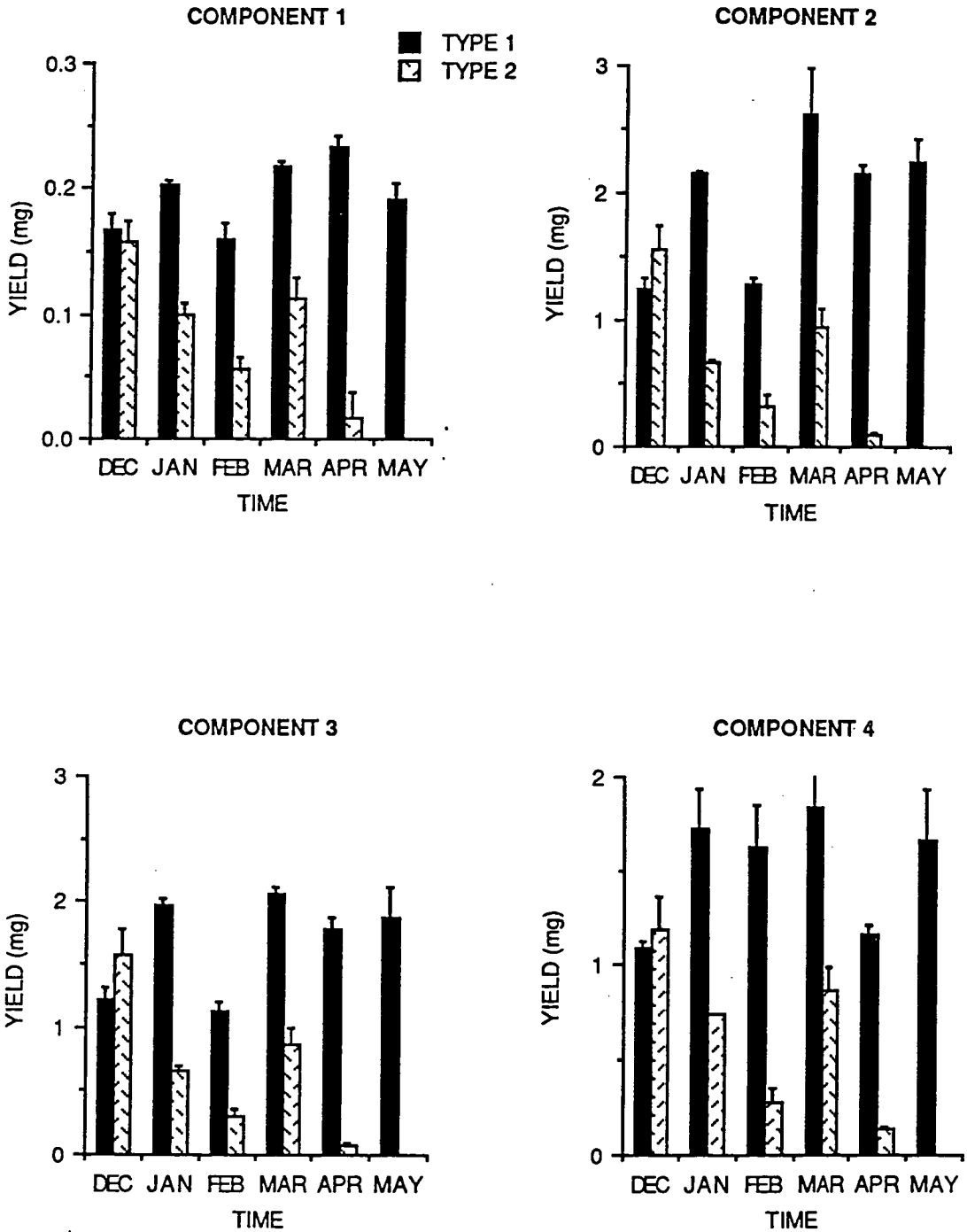


FIGURE IV.5.11

SEASONAL VARIATION OF ESSENTIAL OIL COMPONENTS

ELEPHANT PASS

Component 4 (β-eudesmol, retention time 18.03 min)

The level of β-eudesmol is the same in December for both leaf types. It then increases in type 1 leaves, and remains constant. In type 2, it decreases with the approach of senescence.

The behaviour of the various components can be compared in different oils. For instance, bicyclogermacrene was monitored in all three oils. It seems that the production of bicyclogermacrene is fairly constant in type 1 leaves. It falls to zero at senescence in type 2.

Germacrene-D was seen in EP and BU. In BU it decreases steadily in both leaf types. In EP, it is constant in type 1. It is possible that the physiologiccal development of the two clones is not the same, and the gradual decrease seen in BU, is yet to occur in EP.

PP and EP both contained β-eudesmol. There was a similar trend in both clones. In type 1 leaves, the level of β-eudesmol increased to a given value, and remained there. Again, developmental differences between clones may be affecting the timing of the observed changes.

Each of the three chosen clones has an essential oil which can contain components that are stable and do not vary in percentage concentration. For instance, component 2 EP varies little in percentage concentration with time. Other components display a seasonal fluctuation, which may be due to transport from mature leaves to developing ones, or due to interconversions of essential oil constituents. This means that the total oil composition changes over the growing season, and slight alterations in essential oil quality may be observed depending on time of harvest.

During the winter months, when growth is not rapid, and essential oil content is fairly constant, it is assumed that the changes in essential oil composition are also not great.

5.2c BUSHY PARK SEASONAL VARIATION TRIAL AROMA COMPARISONS OF EXTRACTS

The solvent extracts of each of the three clones involved in the seasonal variation trial were assessed by taking up a sample on a taper and comparing their odours. The observations made are summarised in Table IV.5.4 below, where the numerical suffix of '1' or '2' refer to the Type 1 or Type 2 leaves, as detailed in section IV.5.2a.

The aroma of EP 2 does not develop in any remarkable way, except that is becomes more characteristic. This corresponds to an increase in bicyclogermacrene (Component 4), see Figure IV.5.13.

EP 1 started to give a characteristic impression in February. The monoterpene components of this oil decrease in importance, with the impact and tenacity probably being most influenced by the β-eudesmol content. This character is developed and preserved in the dryout aroma, maintaining its strength throughout. The level of bicyclogermacrene and germacrene-D are both high, and may exert some influence too, whilst the finer details of the character may be supplied by the other major aroma components.

In December, PP 1 and 2 are very similar. By January the type 2 oil already has less of the lighter notes, becoming woody and heavier than type 1 oil. In February the type 1 oil becomes and remains very characteristic, while 2 is woody. The unknown components may be making a contribution to the complex aroma of PP oil, since, of the components investigated, these showed the largest differences in December between the two leaf types. See Figure IV.5.12.

The oil of BU 1 is characteristic even at the beginning of the trial, as is BU 2. It remains so until May, when the aroma becomes a little heavier in its undertones. BU 2 rapidly loses character, leaving only woody and tobacco-like notes. The changes in concentration of components studied in BU oil are not great and this stable nature is reflected in the aroma of the oil at various times.

	Dec	Jan	Feb	Mar	Apr	May
EP1	green (solvent ext'n smell) ••• characteristic dryout	green, woody, waxy ••• strong, pungent sharp notes leaf litter characteristic dryout ###	EP-like ••	green solvent ext'n smell light non-descript • characteristic dryout ##	some 'green' then light floral-woody •• characteristic dryout #	some green •• clear strong EP leaves ### cudesmol smell strong, lasting sweetening with time
EP2	green light fresh •••	woody musky tobacco sharp •••	EP-like a little woodier •	EP-like with green smell as in EP1 but woodier •-••	EP-like •-•• nothing very distinct clear or strong	
PP1	heavy musky woody •••• papery dryout	PP-like fresh some rank undertones •••• papery dryout	PP-like ••• woody character	warm PP-leafy •••	slightly rancid green then PP-like ••• not as much depth as Mar	slight wood rancid then PP- like leafy very strong persistent sharp clear very characteristic sharper clearer with time •••
PP2	heavy musky woody almost meaty ••••	heavier undertones stronger ••• cheesy sweet sawdust, characteristic	tobacco-like woody ••	woodier unpleasant •• rotting wood	nothing •	
BU1	very BU-like ••• woody floral	BU-like ••••• fresh, bush in field smell of BU	BU-like ••••• fresh lighter than Jan	••••• BU-like fresh true	•••• BU-like light fresh characteristic	•••• BU-like very intense fading leaving heavy notes
BU2	BU-like ••• woody floral pleasant	a little woodier than Dec •••• pleasant	BU-like ••• heavier woodier	••• BU-like heavy,no lift faint, soon dissipates	• traces of BU tobacco- like	

TABLE V.5.4

AROMA COMPARISON OF SOLVENT EXTRACTS FROM THE BUSHY PARK SEASONAL VARIATION TRIAL

6. ESSENTIAL OIL YIELD

6.1 THE ESSENTIAL OIL YIELD FROM PLANTS AT OUSE AND BUSHY PARK TRIAL SITES

The essential oil yield of plants from all six clones was monitored monthly at both the Ouse and Bushy Park trial sites. The samples were collected and processed immediately. The solvent extraction method, detailed in Materials and Methods, was used, which meant that triplicate samples could be run. The inclusion of an internal standard allowed the yield to be estimated from the gc analysis. Solvent extraction was used in preference to steam distillation to retrieve the oil, since it was less time consuming and did not require as much destructive harvesting of plants which were being used concurrently in other trials.

During the 12 months following the August harvest ('87) at Bushy Park, the yield from all clones at Bushy Park and Ouse was determined, and the results have been collated in Figures IV.6.1 and IV.6.2 respectively.

Figure IV.6.3 is derived from the same data, however, it is displayed clone by clone so that direct comparisons can be made between the two sites for each clone.

The original data is presented in Tables IV.6.1 and IV.6.2. In order that some measure of site effects might also be obtained, an analysis of variance (ANOVA), was carried out using the combined data from Ouse and Bushy Park. The ANOVA is shown in Table IV.6.3, along with comparisons amongst all of Site, Clone, Date and percentage yield characters. Using a significance level of $p \geq 0.05$, all the comparisons were significant. Specifically, in terms of the percentage oil yield, there is a difference between the clones, and it seems the site effect also plays a part. The seasonal variation of percentage oil yield is significant, and is represented in the plots of yield versus time in Figures IV.6.1 and IV.6.2, as can the effects of site and clone.

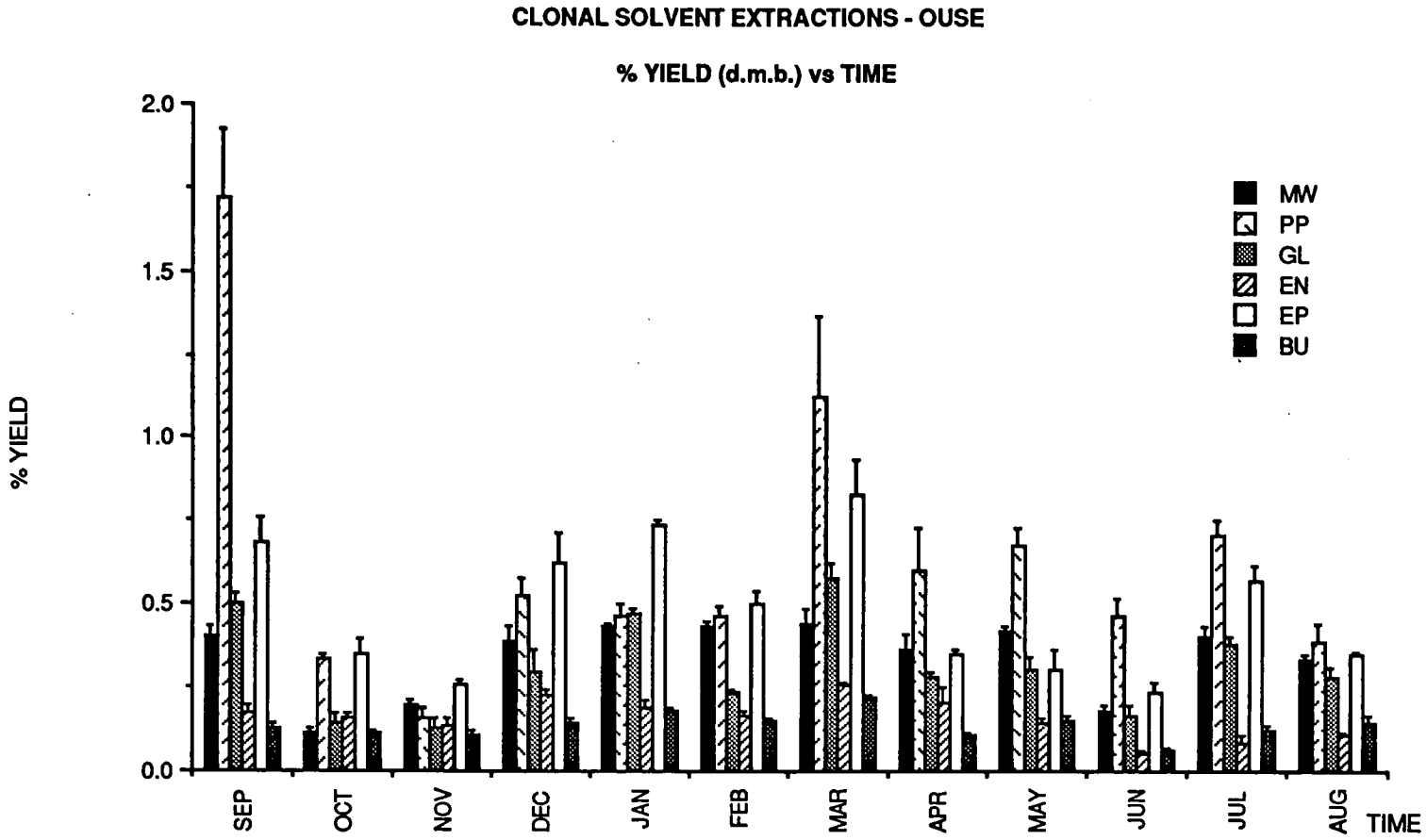

CLONAL SOLVENT EXTRACTIONS - OUSE
% YIELD (d.m.b.) vs TIME
% YIELD
2.0
1.5
1.0
0.5
0.0
MW
PP
GL
EN
EP
BU
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
TIME

FIGURE IV.6.1

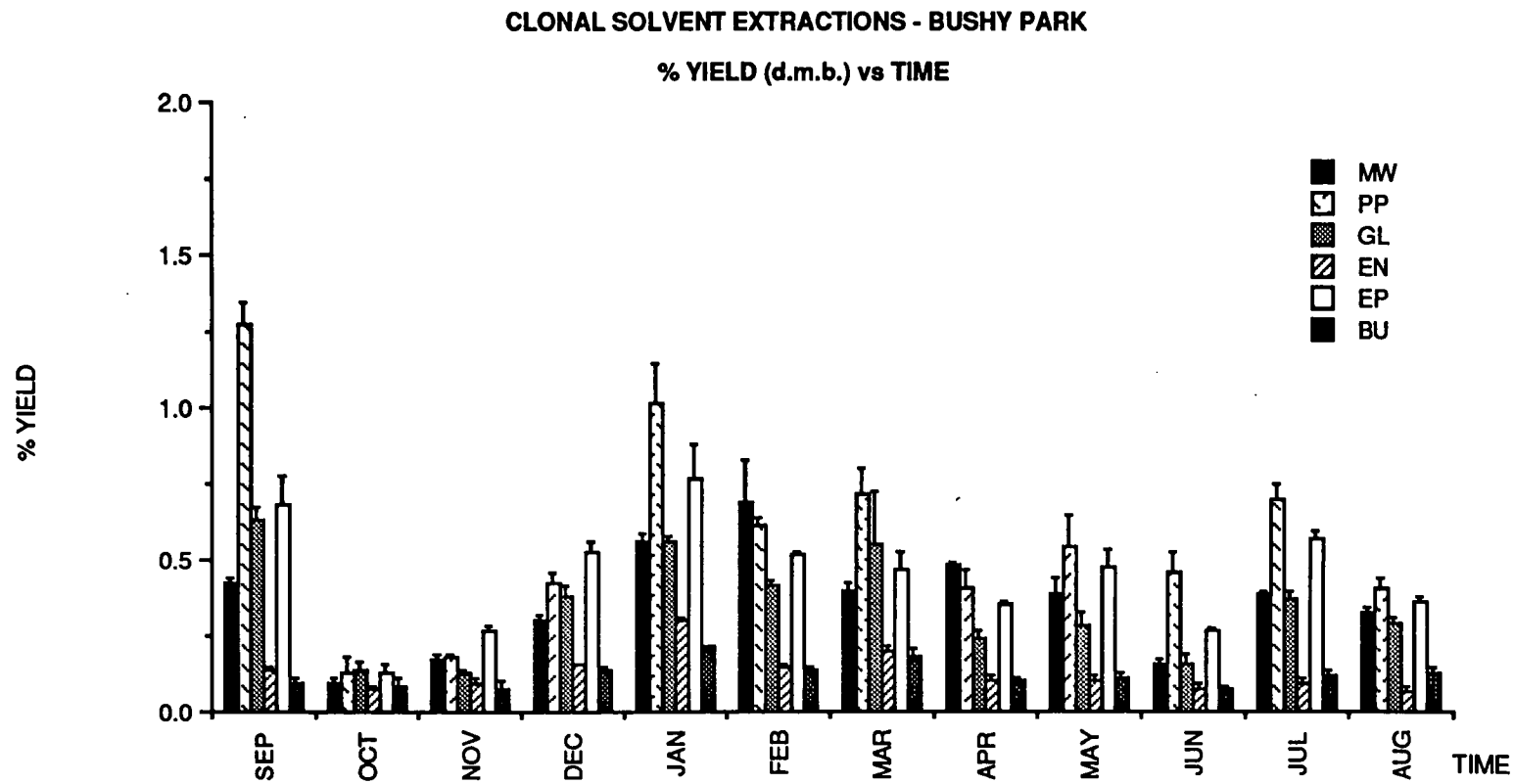

CLONAL SOLVENT EXTRACTIONS - BUSHY PARK
% YIELD (d.m.b.) vs TIME
% YIELD
2.0
1.5
1.0
0.5
0.0
MW
PP
GL
EN
EP
BU
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
TIME

FIGURE IV.6.2

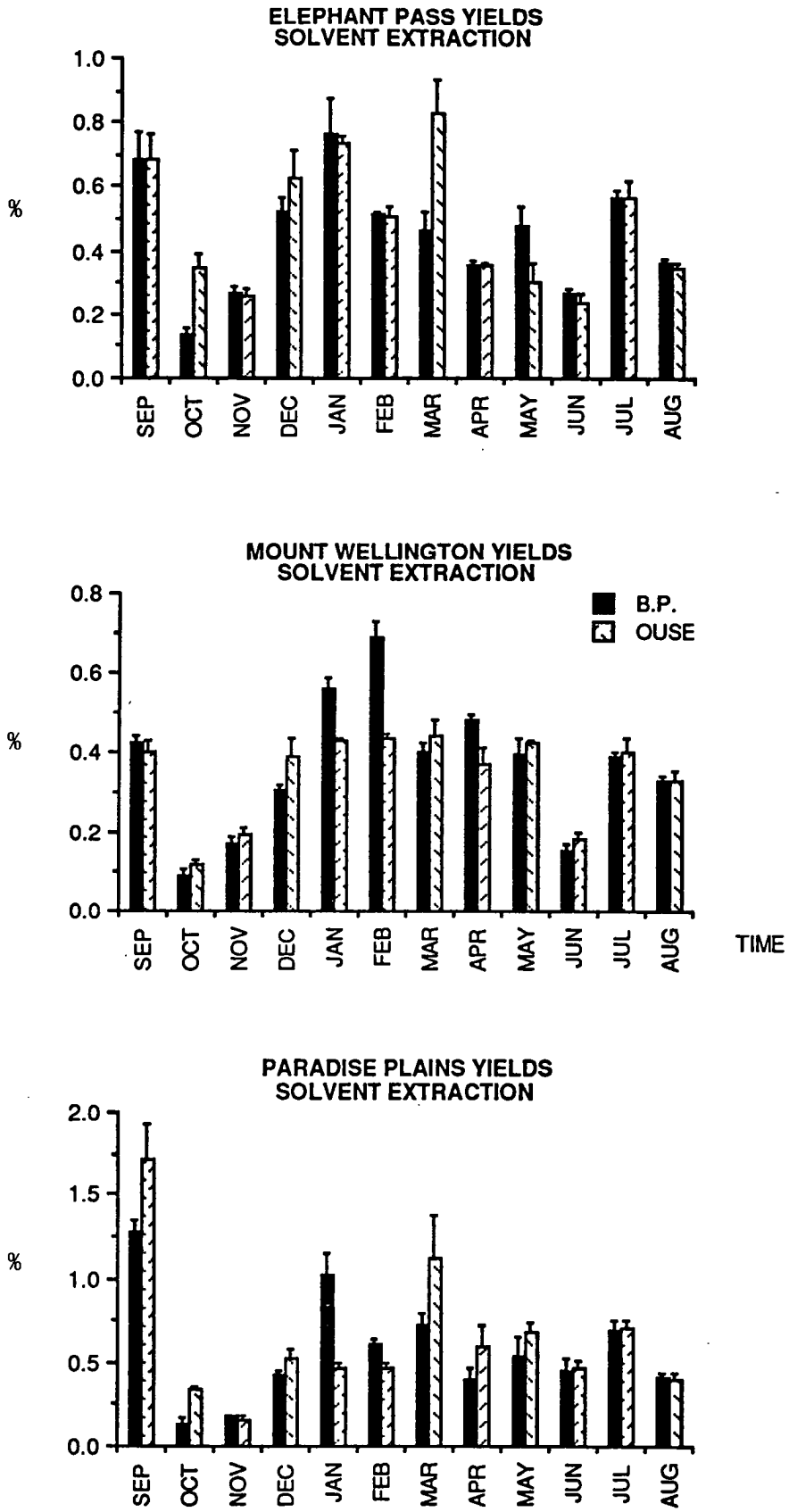


FIGURE IV.6.3a

CLONAL ESSENTIAL OIIL YIELD - OUSE AND BUSHY PARK

% Yield (d.m.b.) vs Time

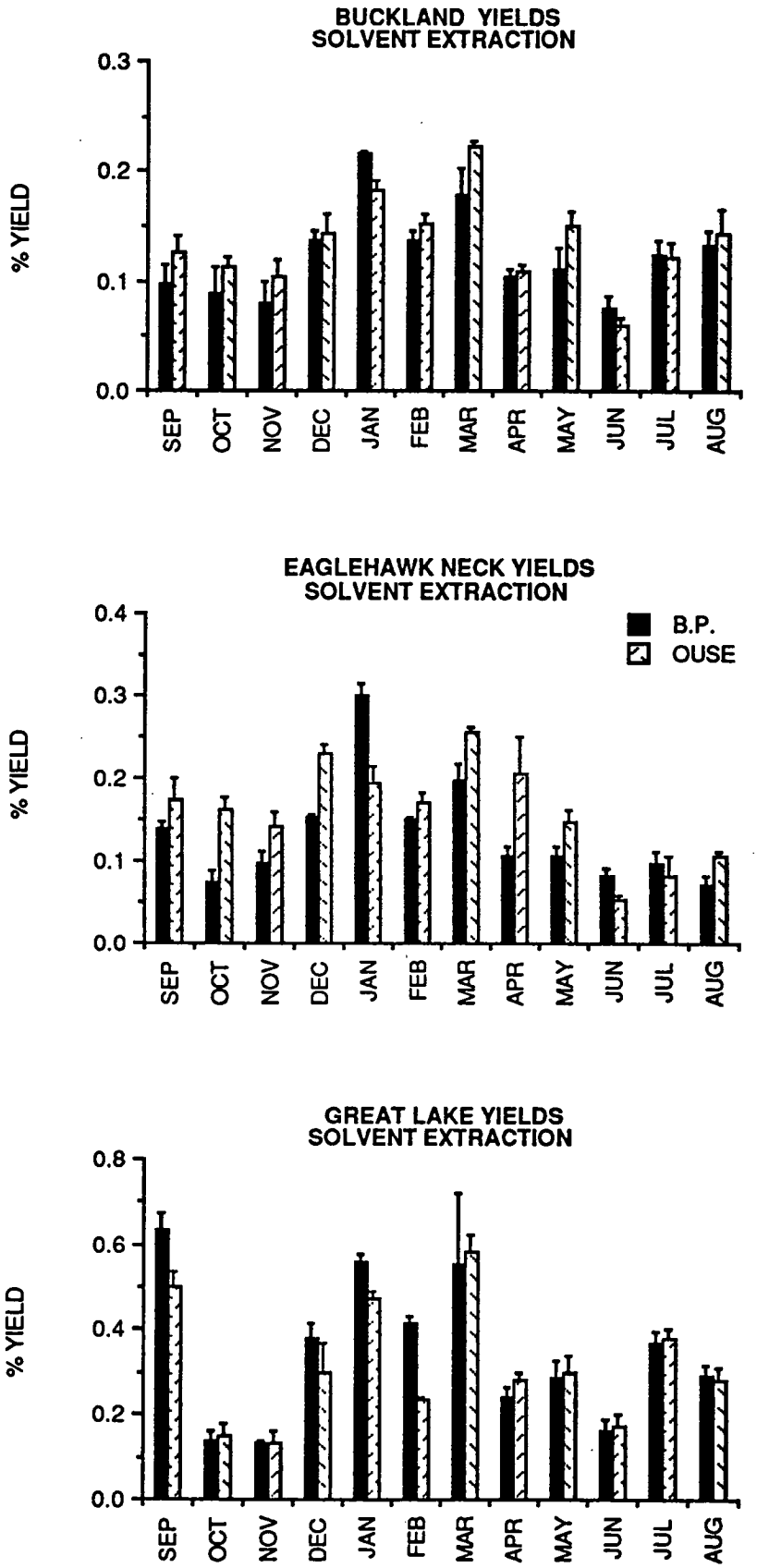


FIGURE IV.6.3b

CLONAL ESSENTIAL OIL YIELD - OUSE AND BUSHY PARK

% Yield (d.m.b.) vs Time

TABLE IV.6.1

PERCENTAGE YIELD OF CLONES AT BUSHY PARK

BY SOLVENT EXTRACTION (d.m.b.)

Standard errors are shown in parentheses

CLONE	MW	PP	GL	EN	EP	BU
SEP	0.4242	1.2789	0.6322	0.1392	0.6841	0.0960
	(.019)	(.070)	(.042)	(.008)	(.090)	(.018)
OCT	0.0911	0.1278	0.1374	0.0740	0.1336	0.0883
	(.017)	(.049)	(.023)	(.013)	(.023)	(.025)
NOV	0.1734	0.1774	0.1316	0.0985	0.2631	0.0785
	(.017)	(.010)	(.008)	(.013)	(.024)	(.021)
DEC	0.3058	0.4190	0.3815	0.1530	0.5221	0.1377
	(.014)	(.039)	(.035)	(.003)	(.041)	(.007)
JAN	0.5610	1.0149	0.5562	0.3011	0.7674	0.2155
	(.025)	(.133)	(.021)	(.013)	(.109)	(.002)
FEB	0.6898	0.6118	0.4171	0.1499	0.5163	0.1367
	(.137)	(.024)	(.015)	(.004)	(.008)	(.008)
MAR	0.4006	0.7182	0.5499	0.1981	0.4619	0.1791
	(.021)	(.083)	(.171)	(.019)	(.063)	(.024)
APR	0.4806	0.4015	0.2420	0.1057	0.3514	0.1047
	(.014)	(.066)	(.023)	(.011)	(.013)	(.005)
MAY	0.3912	0.5418	0.2857	0.1064	0.4755	0.1106
	(.046)	(.109)	(.041)	(.012)	(.059)	(.019)
JUN	0.1515	0.4568	0.1589	0.0811	0.2656	0.0741
	(.021)	(.073)	(.032)	(.011)	(.012)	(.011)
JUL	0.3856	0.6969	0.3698	0.0965	0.5684	0.1237
	(.015)	(.052)	(.025)	(.015)	(.023)	(.013)
AUG	0.3291	0.4080	0.2956	0.0714	0.3629	0.1331
	(.013)	(.032)	(.019)	(.011)	(.014)	(.012)

TABLE IV.6.2

PERCENTAGE YIELD OF CLONES AT OUSE

BY SOLVENT EXTRACTION (d.m.b.)

Standard errors are shown in parentheses

CLONE	MW	PP	GL	EN	EP	BU
SEP	0.4010	1.7210	0.502	0.1750	0.6832	0.1268
	(.031)	(.202)	(.033)	(.026)	(.080)	(.015)
OCT	0.1149	0.3379	0.1477	0.1626	0.3468	0.1130
	(.017)	(.014)	(.028)	(.014)	(.045)	(.009)
NOV	0.1965	0.1619	0.1305	0.1406	0.2557	0.1042
	(.015)	0.027)	0.029)	(.018)	(.021)	(.014)
DEC	0.3907	0.5269	0.2985	0.2295	0.6224	0.1429
	(.042)	(.049)	(.069)	(.012)	(.089)	(.019)
JAN	0.4303	0.4613	0.4707	0.1931	0.7359	0.1828
	(.007)	(.042)	(.016)	(.023)	(.019)	(.010)
FEB	0.4371	0.4652	0.2361	0.1711	0.5052	0.1520
	(.009)	(.030)	(.006)	(.012)	(.033)	(.009)
MAR	0.4439	1.1270	0.5802	0.2558	0.8318	0.2234
	(.039)	(.242)	(.040)	(.007)	(.102)	(.003)
APR	0.3682	0.0420	0.2811	0.2052	0.3529	0.1078
	(.042)	(.130)	(.017)	(.045)	(.011)	(.006)
MAY	0.4220	0.6758	0.3015	0.1478	0.3025	0.1490
	(.010)	(.057)	(.040)	(.014)	(.060)	(.015)
JUN	0.1809	0.4666	0.1701	0.0544	0.2351	0.0605
	(.020)	(.051)	(.029)	(.005)	(.033)	(.006)
JUL	0.4024	0.7047	0.3814	0.0810	0.5690	0.1206
	(.030)	(.049)	(.022)	(.024)	(.049)	(.014)
AUG	0.3314	0.3908	0.2825	0.1059	0.3475	0.1437
	(.022)	(.047)	(.026)	(.005)	(.010)	(.021)

TABLE IV.6.3

ANOVA OF PERCENTAGE OIL YIELD BY SOLVENT EXTRACTION FROM CLONES AT BUSHY PARK AND OUSE TRIAL SITES

Source	d.f.	SS	MS	F-test	p
Between subjects	143	529.7	3.7	0.334	1
Within subjects	432	4791.2	11.1		
treatments	3	3139.7	1046.6	271.8	0.0001
residual	429	1651.6	3.85		
Total	575	5320.9			

CLONE vs SITE	24.936 *
CLONE vs DATE	58.107 *
CLONE vs % YIELD	62.313 *
SITE vs DATE	155.853 *
SITE vs % YIELD	8.344 *
DATE vs % YIELD	236.322 *

* significant using Scheffe F-test, $p > 0.05$

6.2 ODOUR IMPRESSIONS OF OLEARIA CLONE ESSENTIAL OILS

The six Olearia oils from the 1988 harvest were assessed by an experienced independent flavorist. His notes are presented below as an indication of the odour impressions presented by the oils under study:

EN - strong, diffusive green metallic note; fruity in direction of lentisique, warm
EP - fresh estery fruity with a dry spicey backnote
GL - floral green marigold tagette, strong lavender impact, linalyl acetate, peachy
MW - strong fruity tomato DMS type with a dry green note and light floral
PP - warm spicey (spice bud) with fresh note
BU - fruity metallic aldehyde, green vegetable with a cooked note, cooked tomato

A personal impression of the six types of oil are given below:
1988 HARVEST AT BUSHY PARK

EN - strong, papery, disinfectant, green beans, vegetable floral, grassy, bushy
EP - sassafras, moss, light, green, fruit salad with passionfruit, orange, tropical fruit, apple, apricot, sweet
GL - lemon, light, floral, pollen, honey, sweet, cloves
MW - lemon, bush, moss, light fresh spicey, floral 'pink', kitchen, basil, thyme, fruity, apples, sour
PP - heavy, biting, eucalypt, mouth-watering, baked apples, plums
BU - green beans, wet wood, grassy, fruity, acidic, sweet, caramel

1988 HARVEST AT OUSE

EN - woody, fruity, heavy, biting, zinc cream ointment, cloves but not sweet, hospital

EP - woody, biting, green, light, sophisticated

GL - leafy, nettles, spicey, mango, old cooking oil, sharp, sweet, oily-metallic

MW - light, spicey, fruity, biting, woody, bushy, fresh

PP - disinfectant, floor cleaner, linament, bandages, wet wood

BU - mint, light, citrus, woody, bush, pickling juice, spicey, fruity with acid note

1987 HARVEST AT BUSHY PARK

EN - citrus, lemonade, pepperminty, pink sweets, bright, sweet

EP - heavy woody, biting, warm

GL - light, fresh, eucalypt, bush, green, sweet

MW - lemon, spicey, light, sweet fresh, herbs

PP - spicey, light, tobacco, minties, peppery, fresh, lime, green, kitchen cupboards

BU - heavy, sweet, spicey, sharp, acid, woody, anise

6.3 VARIATIONS IN A MAJOR OIL COMPONENT DURING SUMMER

Solvent extraction data was used in the following procedure, applied to the six clones. The mean percentage total peak area was tabulated for bicyclogermacrene, a component which occurs in all six oil types. Since the maximum oil yield occurs in the summer months, the proportion of bicyclogermacrene was monitored over this period, namely from December to March. The bicyclogermacrene content of the oil is shown in Table IV.6.4, and was obtained by determining the peak area as a percentage of the total peak area by gc analysis.

TABLE IV.6.4

PERCENTAGE TOTAL PEAK AREA OF BICYCLOGERMACRENE FOR THE SIX OLEARIA CLONES OVER SUMMER

	DEC	JAN	FEB	MAR
MOUNT WELLINTON				
BUSHY PARK	42.9	31.5	32.8	33.5
OUSE	38.8	31.7	38.0	38.0
PARADISE PLAINS				
BUSHY PARK	14.4	13.6	12.3	10.9
OUSE	13.2	14.7	12.6	15.0
GREAT LAKE				
BUSHY PARK	22.1	30.5	31.9	32.0
OUSE	30.6	30.3	37.9	37.3
EAGLEHAWK NECK				
BUSHY PARK	38.8	31.8	39.4	42.6
OUSE	28.9	39.1	33.2	57.1
ELEPHANT PASS				
BUSHY PARK	23.3	26.2	24.7	22.3
OUSE	28.2	25.8	26.4	24.9
BUCKLAND				
BUSHY PARK	60.7	58.8	61.9	51.7
OUSE	65.1	59.6	59.0	60.2

These percentage concentration means were plotted against time, and are shown in Figure IV.6.4. The ANOVA for the combined data is shown in Table IV.6.5.

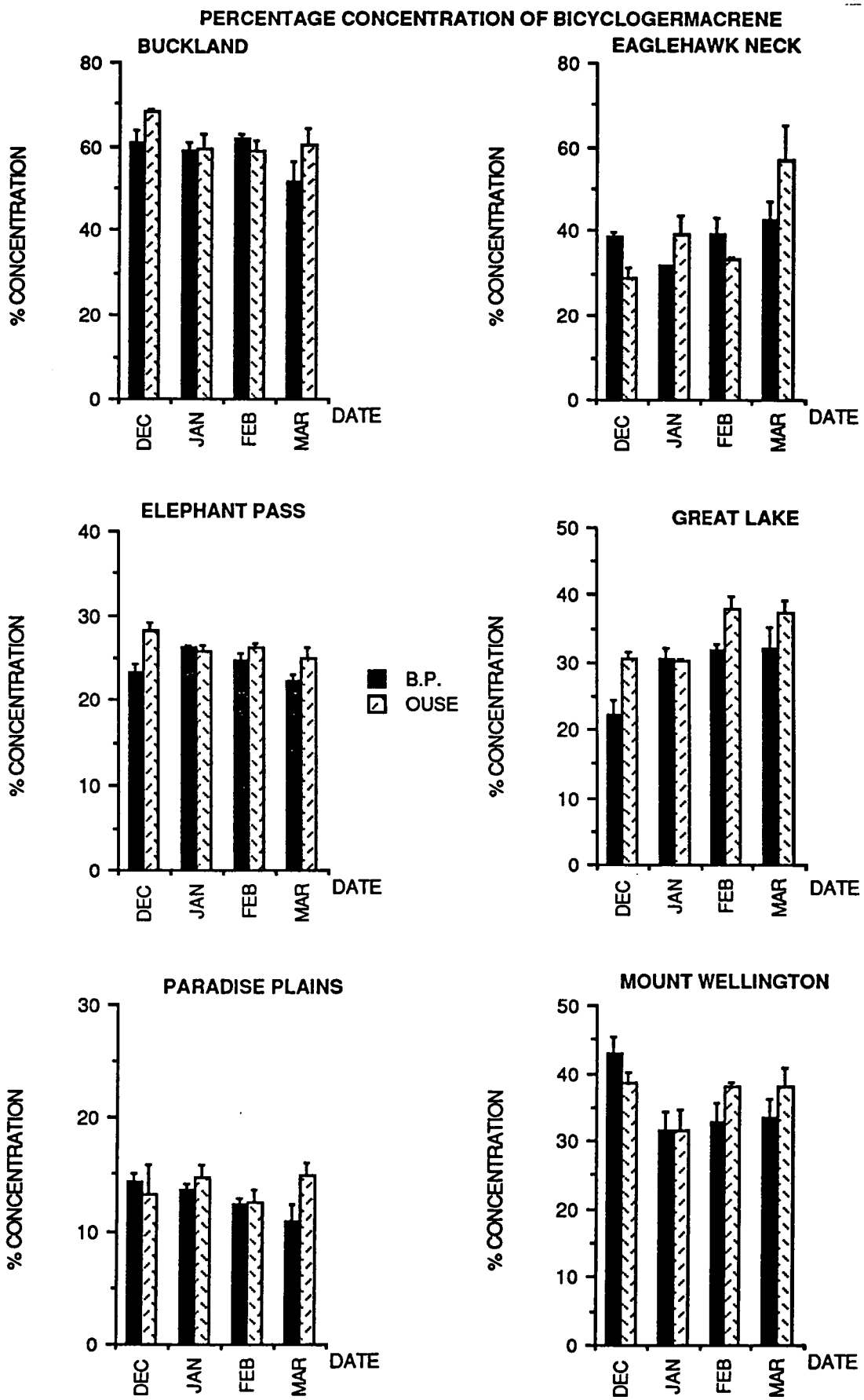

PERCENTAGE CONCENTRATION OF BICYCLOGERMACRENE
BUCKLAND
EAGLEHAWK NECK
ELEPHANT PASS
GREAT LAKE
PARADISE PLAINS
MOUNT WELLINGTON
% CONCENTRATION
DATE
DEC
JAN
FEB
MAR
B.P.
OUSE

FIGURE IV.6.4

TABLE IV.6.5

ANOVA OF MEAN PERCENTAGE CONCENTRATION OF BICYCLOGERMACRENE IN CLONES FROM BUSHY PARK AND OUSE OVER A FOUR MONTH PERIOD

Source	d.f.	SS	MS	F-test	p
Between subjects	47	4393.7	93.5	0.198	1
Within subjects	96	45350.8	472.4		
treatments	2	36788.9	18394.5	201.9	0.0001
residual	94	8561.9	91.1		
Total	143	49744.5			

SITE vs DATE	0.132
SITE vs % CONC.	155.9 *
DATE vs % CONC.	146.9 *

* significant using Scheffe F-test, $p > 0.05$

The composition of the oil was significantly influenced by both site and date. Thus, the composition of the oil can vary during the growing and harvest season, and with the location of the growing site. At Ouse, the MW, GL and EP oils all produced more bicyclogermacrene than at Bushy Park. This effect seems to be more pronounced towards the end of the season, that is, February and March.

Oil quality in MW may be related to the amount of liguloxide present in the oil, since this component has a spicey aroma. The quality of oil may be influenced directly by the amount of liguloxide, and indirectly by the level of bicyclogermacrene and/or other compounds.

6.4 COMPARISON BETWEEN OIL YIELD FROM STEAM DISTILLATION AND SOLVENT EXTRACTION

A small-scale investigation was undertaken to attempt to correlate the percentage oil yield as produced by steam distillation of fresh herb material, with the yield obtained from solvent extraction.

The Mount Wellington clone was used for this trial; sufficient material was collected to run triplicate samples. The standard methods for steam distillation, solvent extraction and dry matter determination were followed, as detailed in Materials and Methods. The solvent extract was also steam distilled in order to determine the percentage steam volatiles constituting the sample.

The results are summarised in Table IV.6.6.

TABLE IV.6.6

STEAM DISTILLATION / SOLVENT EXTRACTION CORRELATION DATA

(note: d.m.b. = dry matter basis)

DRY MATTER

	Wet Wt.(g)	Dry Wt.(g)	% Dry Matter
1.	18.89	8.35	55.80
2.	22.11	9.69	56.18
3.	26.97	12.10	54.95

STEAM DISTILLATION

	Herb Wt.(g)	Yield (g)	% Yield (wet wt.)	% Yield (d.m.b.)
1.	126.78	0.1639	0.1293	0.2317
2.	104.01	0.0988	0.0950	0.1691
3.	36.25	0.0637	0.1752	0.3189

Mean % Yield (d.m.b.) = 0.2399 $\pm$ 0.075

SOLVENT EXTRACTION

	Herb Wt.(g)	Yield (g)	% Yield (wet wt.)	% Yield (d.m.b.)
1.	104.26	0.6754	0.6478	1.1609
2.	102.42	0.5825	0.5687	1.0123
3.	104.31	0.5277	0.5059	0.9207

Mean % Yield (d.m.b.) = 1.0313 $\pm$ 0.1212

Herb originating from the same source yields 1.03 % oil by solvent extraction and 0.23 % by steam distillation. That is, a solvent extract will yield over four times the amount of material obtained by steam distillation.

STEAM DISTILLATION OF SOLVENT EXTRACT

	Wt.(g)	Yield (g)	% Yield
1.	0.6478	0.0675	11.10
2.	0.5825	0.1273	14.42
3.	0.5277	0.0684	12.96

Mean = 12.83 $\pm$ 1.664

Thus, there is some 12.83% volatile material, that is, components which are extractable at 100 °C, in the solvent extracts produced in this fashion.

The preceeding work suggests that the values obtained for transforming solvent extract yields to estimates of steam yields hold true for all instances. This hypothesis was tested by performing the following experiment. Steam distillation and solvent extraction data were available for a twelve month period from both the Ouse and Bushy Park sites. The yields for each clone were collated in terms of percentage yield on a dry matter basis by steam distillation to solvent extraction. The following average ratios were observed:

CLONE	% YIELD STEAM/SOLVENT
GL	47
PP	44
EN	41
MW	37
EP	33
BU	28

From this evidence it is clear that the clonal yield from the different methods of extraction are not uniform. This is due to the different proportions of non-steam volatile materials and waxes present in and on the leaves of the different clones. For this reason, any comparisons between the two extraction methods will use the percentages derived from the larger twelve month study.

6.4a DISTILLATION OF STANDARD HYDROCARBON MIXTURE

Ten hydrocarbons were mixed, in the following proportions, to constitute the standard hydrocarbon mixture:

	mg	%
n-nonane	104.3	10.43
n-decane	115.4	11.54
n-undecane	110.0	11.00
n-dodecane	107.4	10.74
n-tridecane	102.5	10.25
n-tetradecane	103.8	10.38
n-pentadecane	100.4	10.04
n-hexadecane	105.3	10.53
n-heptadecane	110.7	11.07
n-octadecane	100.4	10.04

This mixture was made up in a 50 ml volumetric flask and filled to the mark with hexane. 20 ml of this solution was placed in the laboratory scale distillation apparatus. At intervals, the distillation head was changed, thus allowing the recovery and weighing of the distillation products. In this way, a table of weight of product (in mg) distilled over time was constructed, and is shown below as part of Table IV.6.7. A further dimension was added to this data by calculating the proportions of the various hydrocarbons present in each time sample. This information is also presented in Table IV.6.7

TABLE IV.6.7

YIELD OVER TIME OF A STEAM-DISTILLED HYDROCARBON MIXTURE

Note: the figures in the body of the table are weights in mg.

	TIME (min)					TOTAL	
HC	10	30	60	90	150	YIELD	%
C 9	17.091	0.032	0.022	0.015	0.012	17.17	82.32
C10	19.140	0.041	0.025	0.027	0.022	19.26	83.43
C11	18.491	0.044	0.017	0.020	0.017	18.59	84.50
C12	18.015	0.058	0.019	0.018	0.018	18.13	84.40
C13	17.408	0.108	0.029	0.026	0.017	17.59	85.80
C14	16.810	0.335	0.057	0.054	0.020	17.28	83.22
C15	15.146	0.811	0.078	0.128	0.022	16.19	80.61
C16	14.469	1.553	0.092	0.175	0.029	16.32	77.49
C17	13.603	2.417	0.191	0.258	0.025	16.49	74.50
C18	11.136	2.879	0.232	0.276	0.023	14.57	72.44

From this information, a graph was constructed showing the percentage recovered of each hydrocarbon, Figure IV.6.5. It is evident that yield decreases both for the very volatile components, and for the less volatile ones. In the first instance, the losses are likely to arise as part of the steam distillation process, due to inadequate condensation of these more volatile constituents, whereas hydrocarbons, from say C_{15} onwards, are not readily volatilised.

6.4b STEAM DISTILLATION, OVER TIME, OF A SOLVENT EXTRACT

In conjunction with the preliminary hydrocarbon steam distillation experiment, a solvent extract sample was also steam distilled and samples collected over time. 3.4345 g of PP solvent extract was used. It was introduced into the distillation vessel as a hexane solution. All distillation products were collected with the aid of a hexane solvent trap. The same procedure was used as for the hydrocarbon standard mixture. Samples were collected at intervals, with a sample of each taken for gc analysis. The gc analyses of the hydrocarbon runs produced cutoff retention times which were used to divide the gc traces of the solvent extract runs into three groups. 0-10 min included components up to C_{12}, 11-15

were C_{13} and C_{14} compounds, while 16-30 covered the remainder. Analysing the gc results in this way gave percentage composition estimates for the three ranges, with respect to distillation time. The results are shown in Table IV.6.8 below, and in Figure IV.6.6.

TABLE IV.6.8

PERCENTAGE OF TOTAL COMPOSITION, OVER TIME, OF THREE RETENTION TIME RANGES OF A SOLVENT EXTRACT STEAM DISTILLATION

	DISTILLATION TIME (min)					
RETENTION TIME	15	30	60	90	150	250
0-10 min	70.863	8.443	2.438	0.884	0.810	16.763
11-15	69.869	18.341	7.642	1.965	2.183	0.000
16-30	10.260	17.159	17.947	18.213	18.237	18.184

The figure shows that there is a rapid initial efflux of components which have a retention time of up to 15 minutes. Compounds which have a higher molecular weight take longer to come over, and this is also shown in the graph. The time required for most of these components to undergo distillation seems to be ninety minutes. Note, however, that the higher boiling compounds are still distilling at a uniform rate even after 250 minutes. Hence, one must say that distillation is not complete. However, in the interest of economical operation, a distillation time of 90 to 120 minutes is required to ensure adequate recovery of oil components.

6.4c STEAM DISTILLATION AND SOLVENT EXTRACTION OF OLEARIA ESSENTIAL OILS

The question of chemical composition of essential oil in relation to the method of extraction was one which received attention in this work. Is the oil different when herb material is solvent extracted or steam distilled? To address this problem, fresh material from each of the six clones was collected. Solvent extractions and steam distillations were performed for all clones by the usual methods. Gas chromatographic analyses were performed on all oils produced, as described in Materials and Methods.

Rather than simply comparing the solvent extracts to the steam

FIGURE IV.6.5

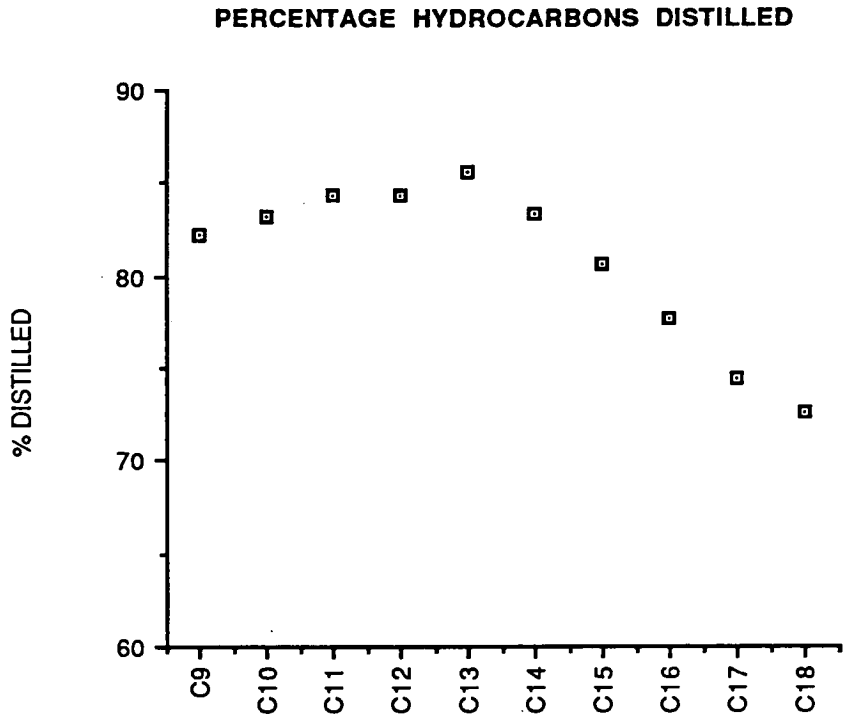
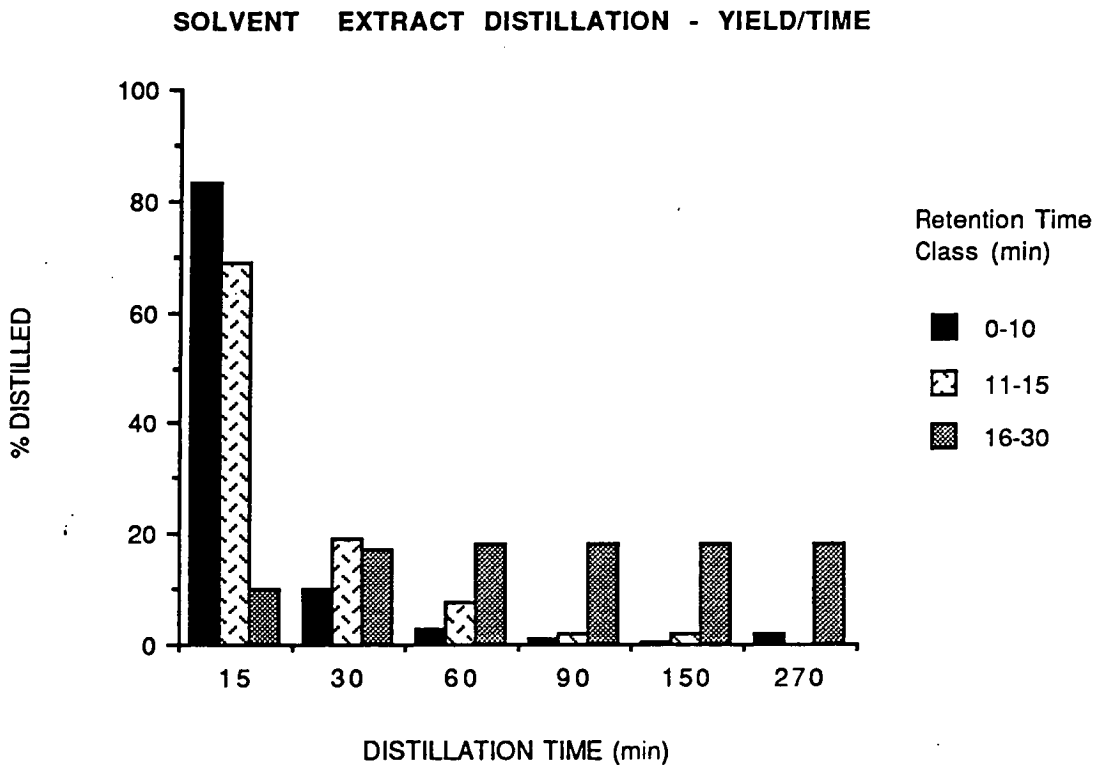


FIGURE IV.6.6



distillation products, the solvent extract was compared with the oil resulting from the steam distillation of the solvent extract. In this way, any discrepancies in oil quality that result directly from the steam distillation process might be detected.

The data was analysed as follows. Major peaks were located in the gc traces for each oil. The peak area of each was expressed as a percentage of the total area of all peaks chosen. From the hydrocarbon standard distillation work, an adjustment factor was applied to each of the steam distillation percentages in order that the volatility of the components was at least partly compensated for. The retention time was used as a guide, in order that the appropriate correction factor for each group (0-10, 11-15 or 16-30 min), was used. The final parameter by which the steam and solvent products were compared, was then:

(% solvent - % adjusted steam) / % adjusted steam

The changes involved in the steam distillation and solvent extraction procedures are complex, and the above analysis proved inadequate. No definite trends emerged. However, a visual inspection of the gas chromatograms, indicated that steam distillation affected few of the components. However, there is one obvious exception, that presents itself prominently in all the oils. This is the component with a retention time of 15.20 minutes. In all cases, this compound occurs in greater concentrations in the steam distilled product than in the solvent extract. In the oils examined, it ranges from 0.6% (in EP) to 8.1% (in GL). Unfortunately, however, its identity remains unknown.

Apart from this one exception, there is no clear evidence that any major differences occur in oil quality as a result of steam distillation.

The solvent extraction procedure may also bring about changes in the oil. By comparing the chromatograms of the steam distillation product with that of the distillation of solvent extract, any differences could be attributed to the solvent extraction process. An example is presented, for GL oil, in Table IV.6.9.

TABLE IV.6.9

COMPARISON OF COMPOSITION OF SOLVENT EXTRACT WITH STEAM DISTILLATION PRODUCT OF SOLVENT EXTRACT

Note: the numbers in the body of the table are percentages; R.T. = retention time

R.T. (min)	DISTILLATION OF SOLVENT EXTRACT	SOLVENT EXTRACT
5.64	0.78	0.35
7.34	2.14	2.75
9.37	0.24	0.42
13.88	0.80	0.59
14.21	0.37	0.34
14.54	1.28	0.00
14.77	31.35	27.28
15.21	7.77	5.64
15.45	1.60	1.31
15.59	0.86	0.60
15.86	0.46	0.33
16.03	4.32	4.89
16.15	1.08	0.71
16.37	17.31	15.34
16.53	2.08	2.35
16.72	0.71	0.51
16.92	1.48	1.12
17.01	0.47	0.45
17.89	18.08	27.57
18.06	1.68	1.09
18.21	0.78	0.38
18.43	1.09	0.95
18.80	0.27	0.33
19.04	1.50	2.04
19.11	1.24	1.80
19.72	0.34	0.86

The above comparison shows that the yield of components both below 15 min and above 17 min is higher in the solvent extract. The compounds that have a retention time between these values appear in the same proportions in either extract. The observed discrepancies may be attributed to a number of factors. A small amount of volatiles may be lost during the steam distillation procedure. In addition, volatiles may be lost when the solvent extract is dried using a rotary evaporator. The other major influence is the increased amount of higher boiling compounds which are extracted by using solvent.

In the literature, examples may be found where chemical transformations occur as a result of solvent extraction. However, they are most prevalent with extraction systems that use either alcohols or acetone (Koedam A., 1987), and are unlikely to be

occurring in this instance.

The determination of changes during solvent extraction, like those during steam distillation, is complex. Elucidation could only come through a further series of experiments.

6.5 PRE-DISTILLATION DRYING

The effect of partially drying the herb before distillation was briefly investigated since the literature cites that this practice facilitates the removal of the oil from the foliage by steam. A sample of MW, with a moisture content of 52.8%, was taken from one bush, sufficient to perform steam distillations on both fresh and dried material. The dried sample was air dried at room temperature to a moisture content of 33.7%. The distillations were carried out using the small still in the laboratory (as described in Materials and Methods), for two hours. The resultant yields were recorded and are presented below in Table IV.6.10.

TABLE IV.6.10

OIL YIELD FROM FRESH AND PRE-DRIED PLANT MATERIAL

	FRESH		
	WEIGHT (g)	OIL YIELD (g)	% YIELD
'FRESH'	251.7	0.0904	0.036
'DRIED'	248.7	0.3044	0.122

Thus, increases in yield may be expected with pre-distillation drying of the herb material. This is due to the relationship between ease of essential oil removal and the percentage dry matter. It seems that when the plant is at or near full turgor, the essential oil is not so easily freed from its containing cells. Weakening of the cellular structure through dehydration facilitates the release of oil.

7. HARVEST OF TRIAL PLOTS

The growth trial area at Bushy Park was harvested twice during the time of this study. The first harvest took place in August 1987. At this time, all trial plants were cut to a height equal to one quarter of their height. This regime was considered to be appropriate on the basis of observations made on the the propensity of Olearia to initiate regrowth when existing growth is damaged or removed.

The second harvest incorporated the sites at both Bushy Park and Ouse, and took place over the period of late May to July, 1988. Harvesting and processing were time consuming processes, so only two clones were cut from each site, in successive batches. The total harvesting procedure took several months to complete.

The data from the harvests was used in the calculation of projected yields per hectare of Olearia essential oils.

7.1 COMPARISON OF SMALL SCALE AND LARGE SCALE DISTILLATIONS OF ESSENTIAL OIL

I. 1987 HARVEST

The Bushy Park harvest in August 1987 was necessary to keep the plot from becoming overgrown. The Ouse site, at this stage, was still manageable, so it was decided not to harvest there until the following year.

The harvest was carried out by hand, with material from each clone processed separately, and the yield from each of the four rows in the plot being recorded individually.

The harvested material was steam distilled in two ways. The bulk was processed at the Horticultural Research Centre, by way of the semi-commercial unit located there. A small sample from each clone was steam distilled in the laboratory, using a small-scale glass still, as described in Materials and Methods.

The yields from the two procedures are given in Table IV.7.1.

TABLE IV.7.1

PERCENTAGE ESSENTIAL OIL YIELD FROM BUSHY PARK OLEARIA CLONES; BULK AND SMALL SCALE DISTILLATIONS

(Percentage yields are on a dry weight basis)

CLONE	YIELD (g) (BULK)	% YIELD (BULK)	% YIELD (SMALL SCALE)
GL	14.3	0.30	0.35
MW	7.9	0.11	0.19
PP	7.5	0.22	0.64
EP	4.8	0.05	0.20
EN	3.9	0.02	0.09
BU	1.3	0.01	0.07

In all cases the yields obtained from the large distillation apparatus were lower than those obtained from the small still. This is explained by the presence of large amounts of less volatile sesquiterpene alcohols which distill slowly. The oils most likely to be affected by this phenomenon are the PP and EP oils. From the table above, it is indeed the oils from these two clones that are affected most.

In addition, the collection vessel was not as efficient as the glass apparatus from the small still. Consequently, a larger version of the laboratory still unit was constructed for use with the semi-commercial still. The recovery of oil was improved with the use of this collector. It had a small surface area (2 cm diameter) to which oil could cling. By comparison, the large 'bell jar' vessel that was originally used was some 25 cm in diameter. The oil tended to form a thin slick on the surface of the water and adhered to the walls, hampering recovery.

II. 1988 HARVEST

During the May to July months of 1988, both the Ouse and Bushy Park trial sites were harvested. The methods and apparatus used were identical to those used during the 1987 harvest. Table IV.7.2 contains the percentage oil yields derived from the material harvested and processed either by the semi-commerical scale still, or the small scale glass still.

TABLE IV.7.2

PERCENTAGE OIL YIELD FROM OUSE AND BUSHY PARK OLEARIA CLONES; BULK AND SMALL SCALE DISTILLATIONS

(Percentage oil yields are on a dry weight basis)

CLONE	YIELD (g) (BULK)	% YIELD (BULK)	% YIELD (SMALL SCALE)
GL			
B.P.	14.9	0.20	0.29
OUSE	12.8	0.19	0.25
MW			
B.P.	5.2	0.04	0.10
OUSE	11.2	0.10	0.13
PP			
B.P.	13.9	0.18	0.45
OUSE	1.3	0.02	0.49
EP			
B.P.	16.1	0.12	0.13
OUSE	7.4	0.09	0.12
EN			
B.P.	7.3	0.04	0.06
OUSE	10.3	0.04	0.04
BU			
B.P.	8.0	0.05	0.07
OUSE	9.7	0.08	0.09

As in 1987, the harvest yields in 1988 from the large scale distillation unit are consistently lower than those obtained from the small still. The percentage yield may be expected to decrease with a scaling up of the procedure, due to larger surface areas, losses within the system and/or incomplete release of oil from a compressed charge. However, precautions can be adopted to minimise the losses which occur purely as a result of operational or technical factors. These would include the maintenance of equipment to avoid leakage and careful loading of the distillation vessel.

An analysis of variance was performed on the data in Table

IV.7.2 (Appendix E), which shows that overall, the total difference between the percentage yield from the bulk distillation is not significantly different (at 95%), from the percentage yield using the laboratory scale still.

The ANOVA also points out the fact that there is a significant site effect, as well as the clone effect.

7.2 COMPARISON OF OILS FROM HARVEST

Essential oils were obtained from all six clones of Olearia using both the laboratory and the semi-commercial distillation units. This enabled a comparison between the oils derived from the larger still and those from the small laboratory still.

The gas chromatographic analyses showed some differences in the amounts of particular components present in oils produced by the two stills. Consequently, the values of percentage composition of major components in the six oils were tabluated for comparison. These are shown in Table IV.7.3.

TABLE IV.7.3

DIRECT COMPARISON OF PERCENTAGE MAJOR OIL COMPONENTS IN OILS DERIVED FROM THE SEMI-COMMERCIAL STILL, THE LABORATORY STILL AND SOLVENT EXTRACTION

(1988 Harvest)

		SEMI-COMMERCIAL		LABORATORY		SOLVENT
CLONE	CODE	BUSHY PARK	OUSE	BUSHY PARK	OUSE	EXT'N
MW	8	26.9	24.4	36.3	24.2	35.5
BU	8	39.9	47.3	61.0	49.4	58.0
EP	8	29.3	20.4	17.6	10.9	23.7
	12	15.2	21.7	30.5	34.6	24.0
EN	7	9.8	16.4	17.9	11.4	18.2
	8	32.8	43.9	31.7	34.3	37.5
PP	8	11.2	10.7	12.1	10.5	11.1
	12	22.4	23.4	14.0	22.0	19.4
GL	6	28.7	36.1	44.7	34.8	33.3
	8	17.5	19.4	17.7	18.4	38.9

The major components are identified with a code number:

6 = caryophyllene

7 = germacrene-D

8 = bicyclogermacrene

12 = β-eudesmol

In general, the figures from the 1987 harvest are in accord

with the 1988 ones. The figures for caryophyllene from GL are not readily interpreted. The value for Bushy Park/semi-commercial is very low. Since less sesquitepene alcohols are produced with the semi-commercial unit, there should be at least as much, if not more, caryophyllene than that from the laboratory still. It is possible that the sample was run with too much steam pressure, so that condensation was incomplete and some volatiles were lost.

It is perhaps more instructive to look at the results in relation to the solvent extractions of samples collected at the same time as the 1988 harvest samples. In that way, we can detect whether there is a distillation effect occurring when the large still is used. These figures are given in Table IV.7.3 under the heading of SOLVENT EXT'N.

From the above table, some observations can be made. There are components in some oils, namely bicyclogermacrene in MW, BU and GL, and germacrene-D in EN, which have a low yield using the large scale apparatus for distillation. Some of these are low yielding even with the laboratory still, GL bicyclogermacrene, for instance. The variations observed can be explained by the fact that:

1) Volatiles are lost during the steam distillation process; much more than with solvent extraction.

2) Heavier components are more difficult to collect by steam distillation since there is the chance that some product will remain in the condenser.

3) The percentage composition of the oil obtained by solvent extraction may differ from the steam distilled oil since more solid substances are retrieved from the sample; volatiles may be lost during the drying down stage of sample preparation.

The salient feature of reliable essential oil production and supply should be reproducibility of product quality. Essential oils have a propensity to vary in composition to some extent despite the producers' best efforts, due to seasonal factors, over which there is no control. It is of utmost importance then, that production techniques be carefully established and maintained so that quality limits are upheld and fall into line year after year. These limits may be set in relation to the major oil components in the oil. In particular, the levels of those components which are responsible for the characteristic nature of each oil. For instance, with MW, the liguloxide and kessane may be more important than that of other major components.

7.3 ESTIMATION OF POTENTIAL CLONAL YIELDS PER HECTARE

The data from the complete harvesting of the trial plots was used to compute potential essential oil yields per hectare. Assuming the same plant spacings are used on a hectare block as in the trial, the following calculations apply:

1 ha = 10,000 m^2.
Plant spacing: 0.5m x 0.5m (triangular configuration) with 0.8 m within rows and 1 m between rows.
Plot area = 129.6 m^2.
There were six clones, thus each clone occupied : 21.6 m^2.
Thus, the weight of material harvestable from each clone is :

weight from 40 plants x (10,000/21.6)
= weight from 40 plants x 463

The weight of fresh material produced by each clone from the Bushy Park and Ouse sites is listed in Table IV.7.4.

TABLE IV.7.4
WEIGHT OF FRESH MATERIAL HARVESTED FOR DISTILLATION FROM THE SIX CLONES AT BUSHY PARK AND OUSE

	WEIGHT OF MATERIAL HARVESTED (kg)	
CLONE	BUSHY PARK	OUSE
GL	13.3	11.9
MW	21.2	20.3
PP	13.7	9.1
EP	22.7	49.1
EN	30.1	13.8
BU	30.0	24.4

Data from Table IV.7.4 was used to construct an ANOVA of weight of harvested material from the two sites. The ANOVA is shown in Appendix F, and the resultant comparisons show that the clone effect is a significant factor in determination of the harvest weight. Interestingly, the site effect is also shown to be significant at 95% by the F-test used. In fact, the effect of site itself may not

be as important as the planting date and previous harvest history. The clonal establishment at Ouse was slower than at Bushy Park, with the exception of the EP clone. Thus, lower yields were recorded for all clones except EP. This particular clone was planted as advanced cuttings and grows vigorously. The high yield at Ouse is the result of uninterupted growth from the start of the growth trial in November '86 to the final harvest in July '88.

The potential weight of distillable material from one hectare of each clone has been calculated and is shown in Table IV.7.5.

TABLE IV.7.5

ESTIMATED YIELD PER HECTARE FOR OLEARIA CLONES

	WEIGHT PER HECTARE (kg)	
CLONE	BUSHY PARK	OUSE
GL	8211	8476
MW	13539	13924
PP	8458	6482
EP	14013	30311
EN	18582	8519
BU	20578	20540

From the weight per hectare figures and the percentage yield (d.m.b.) as determined from the harvest in 1988, the weight of essential oil per hectare can be calculated. The percentages used were the wet weight figures rather than those tabulated in Table IV.7.2, since the herbage production has been calculated on a fresh weight basis. MW (BP) and PP (OUSE) resulted in low percentage yields that are anomalous in comparison with yields from the previous harvest, or the other site. In these cases, the percentage yield from the 1987 harvest was used.

A list of projected yield of essential oil per hectare is shown in Table IV.7.6.

TABLE IV.7.6
PROJECTED ESSENTIAL OIL YIELD PER HECTARE

	ESSENTIAL OIL YIELD/HA (kg)	
CLONE	BUSHY PARK	OUSE
GL	9.03	9.32
MW	6.77	7.66
PP	8.46	6.48
EP	9.81	15.16
EN	3.72	1.70
BU	6.17	8.22

The 1988 harvest was completed after the summer growth had ceased to be vigorous. This was not intentional, but occurred due to the need to maintain an unbroken record of growth measurements over a twelve month period. Ideally, harvest would have occurred towards the end of summer, when the yield of essential oil is highest.

From observations made in the field over the two years that the Bushy Park trial was in operation, it may be feasible to harvest the Olearia crop twice during the year. The first harvest would take place in February, early March. This would yield the bulk of the oil for the year. Substantial growth can still occur after this time, and especially if the autumn is long and the winter is mild, then another harvest during July or August would be worthwhile. The oil from the second harvest would have a slightly different composition than the oil from the first harvest. However, by blending the two products, the volume of oil produced would be increased without sacrificing product quality.

V. GENERAL DISCUSSION

GENERAL DISCUSSION

Olearia phlogopappa, (Olearia), is a woody shrub which exhibits much variation in form. More importantly, chemical composition and quantity of the essential oil it carries in its leaves and stems is also variable. There are differences between populations as well as between individuals in the same population. That is, the morphological traits such as leaf length and width are different from one site to another. In addition, the same characters are significantly different amongst individuals at the same site. Such intra-species variation is not exceptional. In fact, many Australian native plants are polymorphic, though some stabilisation can occur on a geographic basis. For instance, *Grevillea lavabdyRacea* will be true to type from seed collected in one area (Wrigley J.W., 1976).

The process of sifting through the variability in the wild population had several important ramifications. It was discovered that the size of oil glands among plants from a range of localities can be distinctly different, and seem to fall into two groups. Thus, from each site, plants were found which had oil glands that were either significantly larger or smaller than the majority of plants examined. Thus, for instance, at the Great Lake site one plant had significantly larger glands than others. Similar instances were cited for both the Paradise Plains and Elephant Pass sites.

It was initially assumed that the number of oil glands would be related to essential oil yield. However, analysis of the oil yield and oil gland density data showed that oil yield is not closely correlated with either glands per unit area or percentage dry matter. The inclusion of a gland size, or gland filling parameter in the analysis may have aided in establishing the relationship between the oil yield and the pertinent factors.

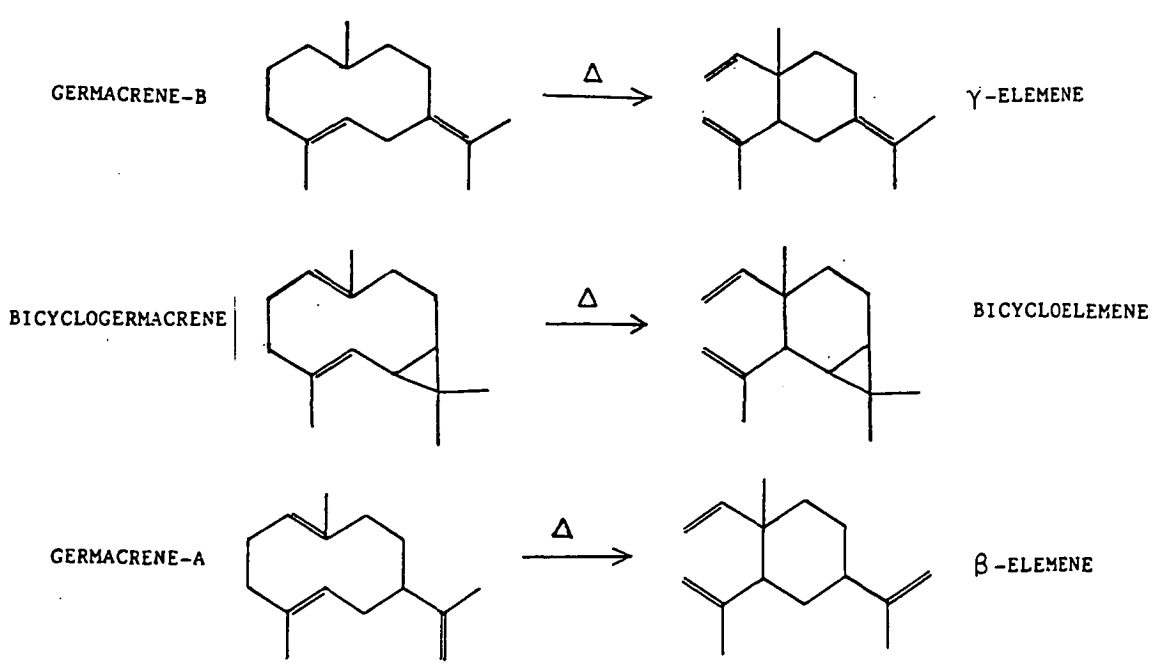
The oil yield is also independent of the concentration of most of the major oil components, except in two instances. Here, one correlation was positive and one negative. This implies that there is no readily accessible parameter which will serve to indicate the potential oil yield of any particular plant. One is obliged to gain this information through direct means, namely distillation of plant samples.

The wild poplulation of Olearia offers much further potential for securing clones which may be commercially acceptable. There is a very broad gene base present which offers valuable material for further breeding work. Work by Pritts M.P. *et al*, (1985), relied on such variability in the population to reveal and harness horticulturally desirable traits.

Six clones, EP, EN, PP, MW, GL and BU, representing Elephant Pass, Eaglehawk Neck, Paradise Plains, Mount Wellington, Great Lake and Buckland, respectively, were selected for clonal material. The essential oils of the six clones were examined. The oils obtained from these plants are complex mixtures, with between 50 and 60 major components. Of these, a number were identifed using a combination of techniques for the isolation, identification and/or confimation of structures.

Through the use of Ftir, gc/ms, hplc, gc and nmr, α-pinene, β-pinene, cineole, linalool, α-terpineol, caryophyllene, germacrene-D, bicyclogermacrene, γ-elemene, elemol, spathulenol, α-eudesmol, β-eudesmol, γ-eudesmol, kessane, caryophyllene oxide and liguloxide, were identified. Of these, the major components in the oils are germacrene-D, bicyclogermacrene and γ-elemene, though β-eudesmol is predominant in the EP oil.

The identification of bicyclogermacrene involved distinguishing it from five other compounds. It is known that germacrene-B, γ-elemene, germacrene-A, β-elemene, bicyclogermacrene and bicycloelemene are related as follows, with each relationship being mediated by heat.



The reactions that occur are Cope rearrangements (where two pi bonds are separated by three single bonds). The reactions can readily occur with the heat involved in steam distillation.

Germacrene-B, bicyclogermacrene, Υ-elemene and bicycloelemene have indistinguishable mass spectra. It is now conceded, with evidence from gc retention times and Ftir data, that the peaks that were formerly being assigned as germacrene-B are bicyclogermacrene. This was confirmed by nmr studies.

Similar alterations of essential oil components during distillation have been discussed often in the literature, both heat mediated, as in the comparison of cold-pressed citrus oils and steam distilled ones (Slater C.A. and Watkins W.T., 1964 and Azzouz M.A. *et al*, 1976), and acid catalysed as in the tendency of sabinene to form terpinene-4-ol, α-terpinene, γ-terpinene and terpinolene (Koedam A., 1987).

The decision to purify the essential oil of MW by means of a simple silica gel column proved very useful. This process was used in this work to pre-fractionate steam distilled oils for gc and hplc analysis. A similar method was used by Scheffer J.J.C. *et al.* (1977), for the enrichment of various components in the fractions of mixtures of naturally occurring oxygenated monoterpenes prior to gc analysis.

Three fractions were obtained. The first contained the hydrocarbons, including most of the monoterpenes; the second fraction contained the oxygenated compounds and the last fraction consisted of alcohols. This pre-fractionation step decreased the number of compounds present in the fractions collected. The chromatograms were less cluttered, and components of interest were easier to isolate.

Aromagrams from hplc analysis were constructed for the MW oil. The particular interest in this clone lies with its unusual tomato, spicey, fruity characters. The fractions of the oil that clearly gave these impressions were identified. However, some components that were typically Olearia in character remain unidentified.

The isolation of components in the second fraction was the main priority, since these were the compounds that produced the major aroma impact of the oil. As a result caryophyllene oxide, kessane and liguloxide were isolated and identified. Liguloxide was found

to be the major component with a spicey odour impact. Other components that were identified as a result of hplc separations were bicyclogermacrene, germacrene-D and spathulenol.

Each oil has its characteristic combination of compounds. In this study, only the major components were considered when looking at oil quality, even though it is widely documented that the odour impact of minor components may be largely responsible for an oil's distinctive notes. For instance, in orange oil, strong odour components are present in an area of the chromatogram where no signal occurs under normal experimental gc conditions (Lamparsky D., 1987).

Olearia oil contains many components in common with other oils of the Asteraceae. For example, oil of *Artemisia dracunculus* L. contains many of the same components as Olearia oils, including α-pinene, β-pinene, limonene, 1,8-cineole, α-terpinolene, linalool, germacrene-D, γ-elemene, caryophyllene oxide and spathulenol (Vernin G. *et al.*, 1987).

Many trends and relationships emerged from the replicated growth trials. The relative growth rates of the six clones, the seasonal variation in growth, oil yield and oil quality are perhaps amongst the most basic and the most important of the these. In order to assess growth, several inter-relating factors were examined.

Growth, or vigour, was seen as the accumulation of dry matter within the plant. After various attempts to correlate dry matter accumulation with several different factors, it was noted that the best estimator of growth was plant height. The stem diameter is also a good indicator, whereas width is not. Plant width is far too dependent on habit and interplant competition to be a continuously valid parameter with which to assess growth.

Percentage dry matter itself is significantly dependent upon clone type, upon the time of year and upon the site of growth.

Another method of estimating the growth was to monitor the rate at which the plant covers the ground surface (as seen from above). The six clones all tended towards 100% cover, but at different rates. At the spacing used, BU was the first to reach 100% cover at Ouse (in 162 days). At Bushy Park, both BU and EN were at 100% at the same time. The growth, as seen in this way, is also well correlated to height increases. Thus, height measurements are a

reflection of growth in terms of both percentage dry matter content and percentage ground cover.

The long term growth trials enabled the growth rates of the six clones to be compared. This revealed that there were significant differences between them. The BU, EN and EP clones produced the most rapid growth (and also the greatest harvest weights). This situation is not directly reflected in corresponding weights of essential oil yield since the percentage yield (on a dry matter basis) differs from one clone to the next. In terms of percentage oil yield, the clones can be loosely ranked as follows: PP>GL>EP>MW>BU>EN. However, with respect to the absolute amount of oil that is actually recovered, the order becomes GL>MW>PP>EP>EN>BU.

At Bushy Park, the growth of plants was observed before and after harvest. The effect of harvest was to reduce the differences between clones. In terms of height, for instance, all six clones appeared more uniform.

The genotypic correlations between pre- and post harvest measures were strong, indicating that, although the differences between clones decreased, the rank order did not. Phenotypic correlation was greatest between the height and increase in height of trial plants. Such a relationship suggests that the greatest growth responses will ensue from large plants. This has implications at the nursery stage of plant production. Plantlets should have a well-developed root system and be actively growing before being transplanted into the field.

The effect of growing site was not determined since the two sites were not established simultaneously. Nor did they have parallel irrigation and weed control programs. The Ouse site was established late, and many plants were lost and replaced before the required number of plants were in place and ready for measurement.

Information from both sites led to the ranking of all clones with respect to height, stem diameter and width. At both sites, with all characters, the ranking was similar, with only minor deviations. The position of the MW clone, for instance, in terms of its width, is a reflection of its prostrate canopy shape. GL is the smallest of the six clones, yet EP has the slowest growth rate.

The work performed on percentage cover increase showed that BU was the most vigorous clone at Ouse and EN at Bushy Park. These

results were supported by the statistical analysis of the height, stem diameter and width measurements made at the two sites. These two clones consistently ranked highest over the period of the trial.

Some consideration was given to the question of whether or not the vigour seen in the trial plants was related to the altitude of the clonal source plant. Evidence was present that with an increase in altitude, there was a greater response from the clone in the trials. On the other hand, one of the most vigorous clones originated from a sandy, sea-level environment. Its response could be attributed to the change in growing medium, rather than any change in altitude. This question does not have a straightforward answer; further work is necessary.

Seasonal variation in growth rate was observed in all clones. The greatest increases occurred early in summer months. A peak in essential oil yield was therefore desirable after this flush of growth.

Some generalisations can be made concerning the seasonal changes that occur in essential oil yield. There is an increase in percentage oil yield, in all clones, during the summer months, from December to March. Some clones, namely MW and GL, showed greater relative increases in yield than others. That is, BU and EN hold a more uniform level of oil content, and the summer peaks are relatively small increases over the amount present during the remainder of the year.

In several clones, (EP, MW, GL), there is the indication that July and/or August bring about a secondary peak in oil yield. Thus, a second winter harvest may by possible on these grounds. From the growth trials, it has been shown that there is some continued growth throughout the year, including the winter season.

As a consequence of a preliminary investigation into the seasonal essential oil variation, the significance of moisture stress was questioned. Glasshouse trials are often limited in their potential for extrapolation into the field situation, and this is especially so in the case of moisture stress experiments. The artificial solution culture situation coupled with the imposition of stress through the addition of PEG is inherently unreliable, as is the measurement of stress by porometery. These effects have been fully considered (Idso S.B. and Allen S.G., 1988, Krizek D.T.,

1985), and the work on Olearia was designed to be relatively simplistic. A larger scale experiment, of longer duration and using other osmotic agents (as a check on toxicity effects), may produce a clearer picture of the trends involved. The uniformity of plants accepted into the experiment could also be a contributing factor to the clarity or otherwise, of the results and the ease with which they can be interpreted.

With Olearia, the trends that emerged from the glasshouse trial showed that moisture stress has little effect on the quantity of oil produced below 4.0 atm.

Since the number of oil glands on Olearia leaves is fixed, the changes that do occur in the quantity of oil is due to incomplete filling of oil glands. Oil quality, however, was noticeably affected at 1.0 atm. It would consequently be prudent to be aware of the possible influences of moisture stress with any work involving the essential oil production in Olearia.

The seasonal variation trials conducted at Ouse and Bushy Park indicated conclusively that the majority of the oil is held in the leaves designated Type 1. That is, leaves of constant physiological age; recently fully expanded. This contrasts to other oil producing crops, such as peppermint, where the oil accumulates in the older leaves (Clark R.J. and Menary R.C., 1981).

In all cases, the amount of essential oil present in ageing leaves decreases with time. Presumably, the oil is reabsorbed from the glands and used in the filling of oil glands in juvenile tissues. Oil accumulation can follow one of two paths in leaves of constant physiological age. For example, in BU and PP there is a peak in oil quantity during February, and again in May. Alternatively, no second peak is observed, as in EP. Also, in leaves of increasing physiological age, the oil content can steadily decline to zero (and senescence) as in BU, or may be interupted by smaller peak in March.

Essential oil quality is also a variable factor in Olearia. Seasonally, there are trends occurring which may be summarised as follows. Some components are not variable in concentration within the oil. An example of this is the level of component 2 and germacrene-D in EP (type 1 leaves). The fate of some components is more complex. Bicyclogermacrene in PP is constant and equivalent for both leaf types from December to February. In March there is a

decrease, and by April type 2 leaves were devoid of bicyclogermacrene. By May, the product seems to have been resynthesised and stored in the younger tissues.

These facts bear significant implications for the harvesting process, since the emphasis is placed on the young growth rather than mature material. A commercial harvesting strategy would consider timing that allowed the maximum amount of vegetative material to be present, bearing maximal quantities of oil.

Olearia plants are amenable to severe pruning to very low levels (ca. 30cm) above the ground, since new shoots are formed readily from adventitious buds, as well as from root material in some clones. When plants are cut low an increase in recovered oil is observed (up to four times that of cutting off only 1/4 of the plant's growth). Less severe pruning does result in larger amounts of material being harvested, however, it is the nature of this material that is critically important if oil yield is to be maximised. Plants that are not cut low develop hard woody stems, which bear old leaves with little oil. In order to encourage the plant to produce as much new growth as possible, a severe harvesting regime is recommended. In this way the younger oil bearing material is available for collection late in summer, and there is a minimum of woody sticks which tend to impair the processing operation.

Of the common commercially grown Asteraceae, there are only two whose foliage is steam distilled for essential oil production. These are Wormwood (*Artemisia absinthium* L.) and Tarragon (*A. dracunculus* L.). Both of these crops are cut or mowed down to leave very short stumps at harvest time. Due to its regenerative abilities, a similar harvest system is envisaged for Olearia.

It is interesting to compare the yields and crop management procedures suggested here with those applied to Wormwood and Tarragon. Wormwood is a much branched perennial, growing over a metre in height. Plants raised from seed or cuttings are planted out in rows 90cm apart, with 45cm between plants. Plants are productive for 7 to 10 years, with the maximum yields occurring after 2 to 3 years. Harvest takes place in the latter part of July (Northern Hemisphere); the plants being cut when in full bloom. The material is tied into sheaves for drying over 24 hours. A delay in harvesting results in decreased yield and quality of oil. Percentage yields range between 0.3 and 0.4%. Tarragon is also a

perennial, which grows to a height of about 60cm. Planting material is derived from divisions from clusters of roots. These are planted in February or March (Northern Hemisphere). A dual harvest system operates with Tarragon; a first harvest takes place in July, with flowers and leaves being taken together, leaving only a very short stump. This grows sufficiently to allow another harvest in September. Harvest is necessary at the beginning of the flowering stage to ensure high quality oil is produced. Oil yields vary from 0.2% to 0.8% (Guenther E., 1949). With Olearia the timing of harvest is different. Maximum yields occur after flowering has finished in February.

With semi-commercial procedures, the percentage yields ranged reached 0.20%, whilst 0.50%, or more, may be possible (d.m.b., 1988 harvest). It must be remembered that these figures apply to a harvest that took place many months after the peak in percentage oil yield had occurred, and therefore, underestimate the full potential yield.

The projected oil yield from Olearia clones ranges from 15 kg/ha (EP Ouse) to 1.7 kg/ha (EN Ouse), though efficient distillation with minimal losses could bring the lower values up considerably, perhaps to about 4 kg/ha. The approximate order of maximum potential oil yield per hectare would be EP>GL>PP>MW>BU>EN.

As is commonly observed, the essential oil yield obtained from the larger semi-commercial scale distillation apparatus is lower than that from laboratory scale equipment. However, statistically, the differences in this instance were not large enough to be significant at $p > 0.05$. It may therefore be quite reasonable to aim for the percentage yields that are obtained with the laboratory scale unit.

The '87, '88 harvests at Bushy Park served to demonstrate the regenerative nature of Olearia. Plants produce an abundance of new young shoots when the old wood is maintained at a height of about 20 to 30 cm.

It is recommended that harvesting takes place in late February to early March, which is the time of maximum oil yield. However, if the composition of the oil is taken into consideration, this time may vary a little, but only to the extent of selecting between January, February or March. With the present state of knowledge, one can only view the situation in terms of the major components of

the oil. For instance, in PP oil, bicyclogermacrene maintains a maximal concentration during December, January and February, falling away rapidly thereafter. The oil of BU has a peak amount of bicyclogermacrene in January, EP in March, and so on. Therefore, the actual time of harvest may be fine-tuned to coincide with these peaks.

One issue that was raised during the processing of harvest material centred on a comparison of the chemical composition of oils derived from the semi-commercial apparatus versus the laboratory still. Some components such as germacrene-D in EN have a very low yield with the large still, whilst others, such as bicyclogermacrene in GL yield low from the laboratory still, compared to a solvent extracted standard. This prompted an investigation of the steam distillation/solvent extraction procedure, as well as the way in which the distillation process effects recovery of various compounds.

The solvent extraction procedure was considered as a possible alternative method of oil extraction, in the event that large compositional changes were occurring during steam distillation. However, no conclusive evidence was found to suggest that major disruptions in oil composition were being produced by the steam distillation method. In fact, it seems that there is a great amount of stability amongst the compounds present. However, there is one obvious exception that was found in all the oils. Under the gc conditions used, this component elutes at 15.20 minutes, and is found in greater concentrations in the steam distilled product than in the solvent extract. In the oils examined, it ranges from 0.6% (in EP) to 8.1% (in GL). Unfortunately, however, its identity remains unknown. As is the case in most commercial essential oils derived by steam distillation, the changes that occur are not without importance. Indeed, the typical, accepted odour of any particular oil may be lost without the changes that occur during the distillation procedure.

The distillation process itself is a complex one. There is an initial rapid efflux of monoterpenes, some of which may be lost due to their volatility. Compounds which have greater molecular weights take longer to distil. Thirty minutes seems to be the required time necessary for most of these components to undergo distillation, though the extent to which compounds are recovered from the plant

material varies. Yield decreases both for low molecular weight components, and for high molecular weight ones. For very volatile compounds it may be difficult to provide adequate cooling for complete condensation. On the other hand, the higher boiling point components are not fully volatilised, and therefore, do not appear as readily in the product collected.

The significantly different yields of the six clones by solvent extraction and steam distillation may be partly explained by the different proportions of heavy solid materials and waxes present in and on the leaves of the different clones. The variation of bicyclogermacrene over the summer months, for instance, is significant with respect to the site as well as clone and date. MW, GL and EP have a higher concentration of bicyclogermacrene when grown at Ouse than at Bushy Park, especially in February and March.

Another factor that was seen to affect the yield of oil was pre-distillation drying of leaf material. The practice of drying plant material prior to processing has been well documented (Heath H.B., 1986), and the increased oil yield thus obtained is suggested to be due to the greater ease with which steam may release the oil from cells disrupted by desiccation.

Solvent extraction has a major disadvantage in that the relatively non-volatile lipophilic substances in the plant material are extracted (Koedam A., 1987). The presence of these fatty oils, waxy materials, flavonoids and coumarins in the extract tend to obscure peaks during gc analysis. As has been observed with Dill by Huopalahti *et al.* , 1981 there is a large increase in the number of peaks in gas chromatograms of solvent extracts compared to steam distillates.

Perhaps one of the most interesting aspects of the chemical study of the Olearia oils has been the chemotaxonomic implications. The traditional classification system has previously placed all of these clones into the one species. However, it is recognised that this species is highly variable : it varies from a small, woody, much-branched shrub 30-40 cm high with leaves having a woolly indumentum on both surfaces, leaves elliptical to oblanceolate in shape 0.8-2.0 cm long, to a medium, densely branched shrub to 2 m with elliptical leaves up to 6.5 cm long, having a woolly indumentum of stellate hairs only on the lower surface. Habitats range from subalpine shrubberies to sclerophyllous forests to the highly

halophyllic communities found on coastal sand dunes (Boyer I.C., 1980, Curtis W.M., 1963). Curtis lists six varieties of *Olearia phlogopappa*, differentiating them on morphological grounds, such as leaf shape and flower structure. These differences are unfortunately not always distinct, since intermediates occur frequently. The advantage of having another criterion by which to separate these plants is obvious.

It seems clear that each of the six clones studied here should be classified under an individual name, since each bears an oil distinctly different from the others. This system may be used for the members of the *O. phlogopappa* series which are odoriferous, but may perhaps be extended to other non-essential oil bearing plants through morphological correlations. For instance, in the Snug Plains a type exists which is morphologically similar to the Buckland type. However, its leaves do not contain an essential oil. Nevertheless, it is placed in the same group as Buckland on its morphological characteristics.

To completely clarify the position, chemical and morphologial features should be linked to enable the classification of plants that are otherwise difficult in that their morphology lies between that of two odour types, but themselves have no oil. Leaf size and floral morphology would, therefore, still be useful tools in determining the status of members of the *O. phlogopappa* species.

All six clones have different growth characteristics, in terms of the amount of leaf and stem material produced, and in what type of canopy it is presented. For instance, MW, PP and GL all have low compact canopies, with small leaves. However, PP and GL are more prostrate than MW. EP, EN an BU all have larger leaf types, but EN and BU produce growth of over one metre in height, which is very dense compared to the lower, more open shape of EP.

The two groups - large leaf and small leaf - also produce essential oils that can be termed simple and complex respectively. That is, GL, PP and MW contain oils that are complex, comprising 50 or 60 components. On the other hand, EP, BU and EN have oils which have few major components.

More data should be compiled about the importance of the minor components of the oils in determining the overall odour impact. The timing of the maximum concentration of these compounds can be used in conjunction with peaks in total oil yield to determine the

optimum time of harvest in order maximise the amount of acceptable oil produced.

This study has emphasised many areas where further investigatory work could be done. Among these are the true nature of the response of Olearia to moisture stress; susceptibility of Olearia to attack by fungal and other diseases; the exact mechanism of distillation of essential oil from plant material; possibility of plant replication through tissue culture; effect of fertilizers and/or sustainable agriculture methods of management; plant spacing effects; and determining definitive standards for essential oil quality.

The differences between the six clones, in essential oil quality, vigour and growth habits have been documented. These may be useful in selecting appropriate clones for individual purposes. In addition, the severity and timing of pruning practices that can be tolerated have been investigated and these indicate the type of harvesting system that should be employed.

In addition, propagation by cuttings was found to be highly successful, with a survival rate of over 90%. It was encouraging to note that these results were easily obtained without the use of additional hormone treatments. Many other Australian endemic species (for instance *Crowea, Boronia, Persoonia* and *Acacia*), show increased rooting responses to added hormones such as indol butyric acid (McIntyre D.K., 1976). Olearia, on the other hand, has an abundance of natural rooting hormones present. A simple system of propagation, such as is possible with Olearia, facilitates its commercialisation.

The information collected in this study will provide a valuable data base for the establishment of commercial Olearia plantations. The components identified in the oils, and their concentrations observed in trial plants, can be used for the determination of harvest and quality standards. A major quality factor in the MW oil is the level of liguloxide, which gives this oil its spicey character.

VI. BIBLIOGRAPHY

Abani M.S.C. and Uzo J.O. (1985) Estimates of genetic and environmental productivity of β-carotene and ascorbic acid in processing tomatoes (*Lycopersicon esculentum* Mill.) in southern Nigeria. *Journal of the Japanese Society of Horticultural Science* 53 (4) 438-443.

Abbott I. and Loneragan O. (1983) Response of Jarrah (*Eucalyptus marginata*) regrowth to thinning. *Australian Forest Research* 13 (3-4) 217-229.

Abou-Dahab A.M. and Abou-Zeid E.N. (1973) Seasonal changes in the volatile oil, cineole and rutin contents of *Eucalyptus camaldulensis* Dehn. and *E. polyanthemos* Schauer. *Egyptian Journal of Botany* 16 (1-3) 345-348.

Albert-Puleo M. (1978) Mythobotany, pharmacology and chemistry of thujone-containing plants and derivatives. *Economic Botany* 32 65-74.

Allen S.G. and Nakayama F.S. (1988) Relationship between crop water stress index and other physiological plant water status indicators in Guayule. *Field Crops Research* 18 (4) 287-296.

Althofer G.W. (1976) Oils, dyes, medicinal and other plant products. *Growing Australian Native Plants. Conference Proceedings.* Namoi Regional Office, University of New England 65-69.

Ammon D.G., Barton A.F.M., Clarke D.A. and Tjandra J. (1985) Rapid and accurate determination of terpenes in the leaves of *Eucalyptus* species. *Analyst* 110 (8) 921-924.

Amoore J.E. (1970) Molecular basis of odour. Springfield, Illinois. C.C. Thomas (Ed).

Antonovics J. and Levin D.A. (1980) The ecological and genetic consequences of density-dependent regulation in plants. *Annual Review of Ecology and Systematics* 11 411-452.

ApSimon J.W., Yamasaki K., Fruchier A. and Chau A.S.Y. (1977) Carbon-carbon bond cleavage in an epoxide. A minor product from the acid treatment of endrin. *Tetrahedron Letters* 41 3677-3680.

Arctander S. (1960) Perfume and flavor materials of natural origin. Published by the author Elizabeth N.J. p. 13.

Azzouz M.A., Reineccius G.A. and Moshonas M.G. (1976) Comparison between cold-pressed and distilled oils through the application of gas chromatography and mass spectrometry. *Journal of Food Science* 41 (2) 324-328.

Beets M.G.J. (1978) Structure-activity relationships in human chemoreception. Information Retrieval Ltd., London.

Birth G.S., Dull G.G, Renfroe W.T. and Kays S.J. (1985) Nondestructive spectrophometric determination of dry matter in onions. *Journal of the American Society of Horticultural Science* 110 (2) 297-303.

Bohlmann F. (1980) New sesquiterpenes from Composites. *VIII th International Congress of Essential Oils. Cannes.* 41-47.

Bohlmann F. and Le Van N. (1978) Neue sesqui- und diterpene aus *Bedfordia salicina. Phytochemistry* 17 1173-1178.

Bohlmann F., Mahanta P.K., Jakupovic J. Rastogi R.C. and Natu A.A. (1978) New sesquiterpene lactones from *Inula* species. *Phytochemistry* 17 1165-1172.

Bohlmann F. and Zdero C. (1978a) Neue furaneremophilane und andere sesquiterpene aus vertretern der gattung *Euryops. Phytochemistry* 17 1135-1154.

Bohlmann F. and Zdero C. (1978b) New furanosesquiterpenes from *Eumorphia* species. *Phytochemistry* 17 1155-1159.

Bohlmann F. and Zdero C. (1978c) New furanoeremophilanes from South African *Senecio* species. *Phytochemistry* 17 1161-1164.

Bohlmann F., Zdero C., King R.M. and Robinson H. (1986) Neue Sesquiterpenlactone und andere Inhaltsstoffe aus *Stevia mercedensis* und *Stevia achalensis*. *Liebigs Annalen Der Chemie* 799-813.

Bohlmann F., Zeisberg R. and Klein E. (1975) ^{13}C NMR spektren von Monoterpenen. *Organic Magnetic Resonances* 7 426-432.

Boyer I.C. (1980) Some aspects of the evolution and taxonomy of Tasmanian Compositae. Honours Thesis, University of Tasmania.

Boyer J.S. (1976) Photosynthesis at low water potentials. *Philosophical Transactions of the Royal Society London* B 273 501-512.

Bradshaw A.D. (1973) Environment and phenotypic plasticity. In: Basic Mechanisms in Plant Morphogenesis. Brookhaven Symposia in Biology 25 75-94.

Brooker M.I.H. and Lassak E.V. (1981) The volatile leaf oils of *Eucalyptus ovata* Labill. and *E. brookerana* A.M.Gray (Myrtaceae). *Australian Journal of Botany* 29 605-615.

Brower K.R. and Schafer R. (1975) The recognition of chemical types by odour. *Journal of Chemical Education* 52 (8) 538-540.

Brud W.S. (1980) The relationship between chemical composition and organoleptic properties of essential oils. Some observations on odour theory. *Dragoco Report* 27 41-53.

Burgess T.I. (1986) A study of some of the factors influencing oil accumulation in *Eucalyptus radiata* var *australiana* in Tasmania. Honours Thesis, University of Tasmania.

Burquez A. (1987) Leaf thickness and water deficit in plants: a tool for field studies. *Journal of Experimental Botany* 38 (138) 109-114.

Carr M.E. (1985) Plant species evaluated for new crop potential. *Economic Botany* 39 (3) 336-345.

Cheesman J.H., Roberts E.C. and Tiffany L.A. (1965) Effects of nitrogen level and osmotic pressure of the nutrient solution on incidence of *Puccinia graminis* and *Helminthosporium sativum* infection in Merion Kentucky Bluegrass. *Agronomy Journal* 57 (6) 599-602.

Clarke J.M. and McCraig T.N. (1982) Leaf diffusive resistance, surface temperature, osmotic potential and CO_2-assimilation capability as indicators of drought intensity in rape. *Canadian Journal of Plant Science* 62 785-789.

Clark R.J. and Menary R.C. (1979a) Effects of photoperiod on the yield and composition of peppermint oil. *American Society of Horticultural Science* 104 (5) 699-701.

Clark R.J. and Menary R.C. (1979b) The importance of harvest date and planting density on the yield and quality of Tasmanian peppermint oil. *American Society of Horticultural Science* 104 (5) 702-706.

Clark R.J. and Menary R.C. (1981) Variations in composition of peppermint oil in relation to production areas. *Economic Botany* 35 (1) 59-69.

Clark R.J. and Menary R.C. (1984) The effect of two harvests per year on the yield and composition of Tasmanian peppermint oil (*Mentha piperita* L.). *Journal of Science Food and Agriculture* 35 1191-1195.

Cotterill P.P. and Zed P.G. (1980) Estimates of genetic parameters for growth and form traits in four *Pinus radiata* D. Don progeny tests in South Australia. *Australian Forest Research* 10 155-167.

Craveiro A.A., Andrade C.H.S., Matos F.J.A., Alencar J.W. and Machado M.I.L. (1986) Essential oils from Brazilian Compostiae. *Journal of Natural Products* 49 (2) 361-363.

Croasmun W.R. and Carlson R.M.K. (Ed.) (1987) Two-dimensional NMR spectroscopy. Applications for chemists and biochemists. VCH Publishers, New York.

Croteau R. and Sood K. (1985) Metabolism of monoterpenes. *Plant Physiology* 77 (4) 801-806.

Curtis W.M. (1963) The student's flora of Tasmania. Part 2. L.G.Shea, Government Printer, Tasmania.

Denissova G.A. (1975) Attempts of classification of terpenoid-containing conceptacles in plants (In Russian) *Botanicheskii Zhurnal SSSR* 60 1698-1706.

Dobson H.E.M., Bergstrom J., Bergstrom G. and Groth I. (1987) Pollen and flower volatiles in two *Rosa* species. *Phytochemistry* 26 (12) 3171-3173.

Dragar V.A. (1984) A preliminary survey of selected species of endemic plants to determine commercial cropping potential for essential oils. Masters Thesis. University of Tasmania.

Dravnieks A., McDaniel H.C. and Powers J.J. (1979) Comparison by twelve laboratories of the odour quality of nine chemicals sniffed from the bottle and as GLC effluent. *Journal of Agricultural and Food Chemistry* 27 (2) 336-346.

Emerenciano V. de P., Ferreira Z.S., Kaplan M.P.C. and Gottlieb O.R. (1987) A chemosystematic analysis of tribes of Asteraceae involving sesquiterpene lactones and flavonoids. [Review *Phytochemistry* 26 (12) 3103-3115.

Erickson R.E. (1976) The industrial importance of monoterpenes and essential oils. *Journal of Natural Products* 39 (1) 8-19.

Fahn A. (1988) Secretory tissues and factors influencing their development. *Phyton: Annals Rei Botanicae (Austria)* 28 (1) 13-26.

Fisher D.B. (1985) *In situ* measurement of plant water potentials by equilibration with microdroplets of polyethylene glycol 8000. *Plant Physiology* 79 270-273.

Formacek V. and Kubeczka K.-H. (1982) Carbon-13 NMR analysis of essential oils. *World Crops: Production, Utilization and Description* 7 (Aromatic Plants) 177-181.

Forsyth C. and Van Staden J. (1983) Germination of *Tagetes minuta* L. I. Temperature effects. *Annals of Botany* 52 (5) 659-666.

Freidrich H. (1976) Phenylpropanoid consitituents of essential oils. *Journal of Natural Products* 39 (1) 1-7.

Garlick B.K. (1977) Quality factors in lavandin. *Perfumer and Flavorist* 2 (4) 25-26.

Gray A.J. (1985) Adaptation in perennial coastal plants - with particular reference to heritable variation in *Puccinellia maritima* and *Ammophila arenaria*. *Vegetatio* 61 (1-3) 179-188.

Greger H., Zdero C. and Bohlmann F. (1986) Eudesman-12,8 -olides and other terpenes from *Artemisia* species. *Phytochemistry* 25 (4) 891-897.

Grill D., Pheifhofer H. and Tschulik A. (1987) Investigations of seasonal variations of Spruce needle compounds in relation to frost hardiness. *Phyton: Annals Rei Botanicae (Austria)* 27 (2) 221-240.

Groweiss A. and Kashman Y. (1978) Xeniculin, xeniophyllenol and xeniophyllenol oxide. New diterpeniods from the soft-coral *Xenia macrospiculata*. *Tetrahedron Letters* 25 2205-2208.

Grub H. (1980) Optical rotation and odour illustrated using examples from the p-menthane series. *VIII th International Congress of Essential Oils. Cannes.* 650-654.

Guenther E. (1949) The essential oils. Robert E. Kreiger Publishing Co. Inc., New York.

Hancock W.S. and Sparrow J.T. (1984) HPLC analysis of biological compounds. A laboratory guide. Marcel Dekker Inc., New York.

Healey P.L., Mehta I.J. and Westerling K.E. (1986) Leaf trichomes of some *Parthenium* species. *American Journal of Botany* 73 (8) 1093-1099.

Heath H.B. (1978) Flavour technology : Profiles, Products, Applications. AVI Publishing Company. Westport. Conn. USA.

Heath H.B. (1986) Herb and spice essential oils : Production, quality and chemistry. In : On Essential Oils. Verghese J.(Ed). S.T.Reddiar & Sons (Ekm.), India. pp 15-121.

Heybroek H.M. (1984) Clones in forestry and in nature. *Arboriculture Journal* 8 275-286.

Hikino H., Hikino Y., Takeshita Y., Shirata K. and Takemoto T. (1963) Structure of kessan. *Chemical and Pharmaceutical Bulletin* 11 547-548.

Hirota H., Tanahashi Y. and Takahashi T. (1980) Structure of a guaioxide-type sesquiterpene from 'San-shion'. *The Chemical Society of Japan Bulletin* 53 785-788.

Huneck S., Janick S., Meinunger L., Snatzke G., Connolly J.D. and Asakawa Y. (1984) Seasonal dependence of the essential oil from *Bazzania trilobata*. The stereochemistry and absolute configuration of (-)-5-hydroxycalamene. *Journal of the Hattori Botanical Laboratory* 57 337-342.

Huopalahti R., Kallio H., Karppa P. and Linko R. (1981) Comparison of two isolation procedures for aroma compounds of dill. In: Flavour '81. pp. 369-376 Ed: Schreier P. Walter de Gruyter & Co., Berlin.

Idso S.B. (1988) Development of a simplified plant stomatal resistance model and its validation for potentially transpiring and water-stressed water hyacinths. *Atmospheric Environment* 22 (8) 1707-1713.

Idso S.B., Allen S.G. and Choudhury B.J. (1988) Problems with porometry: measuring stomatal conductances of potentially transpiring plants. *Agricultural and Forest Meteorology* 43 (1) 49-58.

Ishii H., Tozyo T., Nakamura M. and Funke E. (1972) Microbial transformation of sesquiterpenoids. V. Preparation of 1-epi-liguloxide, the sixth stereoisomer of guaioxide. *Chemical and Pharmaceutical Bulletin* 20 (1) 203-205.

Jakupovic J. Chen Z.-L. and Bohlmann F. (1987) Artanomaloide, a dimeric guaianolide and phenylalanine derivatives from *Artemisia anomala*. *Phytochemistry* 26 (10) 2777-2779.

Jarvis P.G. (1976) The interpretation of the variations in leaf water potential and stomatal conductance found in canopies in the field. *Philosophical Transanctions of the Royal Society London* B 273 593-610.

Juell S.M.K., Hansen R. and Jork H. (1976) Substances first isolated from the essential oil of two Artemesia species I. Spathulenol, an azulenogenic C_{15} alcohol. *Archives of Pharmacy* 309 458-466.

Kellas J.D., Owen J.V. and Squire R.O. (1982) Response of *Eucalyptus sideroxylon* to release from competition in an irregular stand. *Victoria Forests Commission* 29 33-36.

Kester D.E. (1983) The clone in horticulture. *HortScience* 18 (6) 831-837.

Koedam A. (1987) Some aspects of essential oil preparation. In: Capillary gas chromatography in essential oil analysis. pp. 13-28. Eds: Sandra P. and Bicchi C. Huethig.. Heidelberg.

Komae H. and Hayashi N. (1975) Separation of essential oils by liquid chromatography. *Journal of Chromatography* 114 258-260.

Kovats E. (1987) Composition of essential oil. Part 7. Bulgarian oil of Rose (*Rosa damascena* Mill.). *Journal of Chromatography* 406 185-222.

Krizek D.T. (1985) Methods of inducing water stress in plants. *HortScience* 20 (6) 1028-1038.

Kubeczka K.-H. (1973) Separation of essential oils and similar complex mixtures for GC analysis by means of modified dry-column chromatography. *Chromatographia* 6 (2) 106-108.

Kubeczka K.-H. (1984) Progress in isolation techniques for essential oil constituents. *Advances in Medicinal Plant Research Plenary Lecture International Congress.* (32) 197-224.

Kumamoto J., Scora P.W. and Clerx W.A. (1985) Composition of leaf oils in the genus *Parthenium* L. (Compositae). *Journal of Agricultural and Food Chemistry* 33 (4) 650-651.

Lamparsky D. (1987) 'Fingerprints' in essential oil analysis: Aim, results and correlations with sensory values. In: Capillary gas chromatography in essential oil analysis. pp. 155-213 Eds: Sandra P. and Bicchi C. Heuthig., Heidelberg.

Lapierre C. and Monties B. (1984) Photosynthetically C-labelled *Poplar lignans*: ^{13}C NMR experiments. *Holzforshung* 38 333-342.

Lassak E.V. and Southwell I.A. (1980) Survey of Australian flora. *Biological and Chemical Research Institute Research Report.* Department of Agriculture, New South Wales. 43-44.

LeQuere J.L. and Latrasse A. (1986) Identification of (+)-spathulenol in the essential oil of blackcurrant buds (*Ribes nigrum* L.). *Sciences Des Aliments* 6 47-58.

M^{c}Intyre D.K. (1976) Specialist propagation techniques. *Growing Austalian Native Plants Conference Proceedings* Namoi Regional Office, University of New England 15-20.

Maciel G.E., Haw J.F., Smith D.H., Gabrielsen B.C. and Hatfield G.R. (1985) Carbon-13 NMR of herbaceous plants and their components using cross polarization and magic-angle spinning. *Journal of Agricultural and Food Chemistry* 33 (2) 185-191.

Magambo M.J.S. and Waithaka K. (1985) The effect of pruning at different heights on yields, dry matter production and partitioning in clonal tea (*Camellia sinensis*) in Kenya. *Experimental Agriculture* 21 (1) 67-72.

Martin F.W. and Cabanillas E. (1967) Heritability of yields in *Discorea floribunda*. *Tropical Agriculture (Trinidad)* 44 (1) 45-51.

Mathur R.S., Sharma K.K. and Joshi S.R. (1983) Effect of size of shoot cuttings on the growth of *Populus x euramerica* '72/58'. *Indian Forester* 109 (9) 665-674.

Matsuo A., Nozaki H, Kubota N., Uto S. and Nakayama M. (1984) Structures and conformations of (-)-isobicyclogermacrenal and (-)-lepidozenal, two key sesquiterpenoids of the cis- and trans-10,3-bicyclic ring systems, from the liverwort *Lepidozio vitrea*: X-ray crystal structure analysis of the hydroxy derivatives of (-)-isobicyclogermacrenal. *Journal of the Chemical Society. Perkin Transactions 1* 203-214.

Menadue Y. and Crowden R.K. (1983) Morphological and chemical variation in populations of *Richea scoraria* and *R. angustifolia* (Epacridaceae). *Australian Journal ot Botany* 31 73-84.

Mesquita A.A.L., Correa D. de B., de Padua A.P., Guedes M.L.O. and Gottlieb O.R. (1986) Flavonoids from four Compositae species. *Phytochemistry* 25 (5) 1255-1256.

Mitzner B.M. and Mancini V. (1969) Infra-red spectra of oxygenated terpenes. *American Perfumer and Cosmetics* 84 37-46.

Morrison D.A. and Weston P.H. (1985) Analysis of morphological variation in a field sample of *Caladenia catenata*(Smith) Druce (Orchidaceae). *Australian Journal of Botany* 33 (2) 185-195.

Motto M.G. (1987) Techniques of essential oil identification via gas chromatography with capillary columns. *Journal Of Chromatographic Science* 25 (2) 56-59.

Nishimura K., Horibe I. and Tori K. (1973) Conformation of 10-membered rings in bicyclogermacrene and iso-bicyclogermacrene. *Tetrahedron* 29 271-274.

Nishimura K., Shinoda N. and Hirose Y. (1969) A new sesquiterpene, bicyclogermacrene. *Tetrahedron Letters* 36 3097-3100.

Pitarevic I., Kuftinec J., Blazevic N. and Kustrak D. (1984) Seasonal variation of essential oil yield and composition of Dalmation sage. *Journal of Natural Products* 47 (3) 409-412.

Premakumari D. Joseph G.M. and Panikkar A.O.N. (1985) Structure of the bark and clonal variability in *Hevea brasiliensis* Muell. Arg. (Willd. ex A. Juss.) *Annals Of Botany* 56 (1) 117-123.

Pritts M.P., Hancock J.F. and Roueche J.M. (1985) Identifying superior genotypes of blueberry in wild populations. *HortScience* 20 (3) 409-413.

Pushpangadan P., Sobti P. and Khan R. (1975) Inheritance of major essential oil constituents in *Ocimum basilicum* var *glabratum* Benth. (French Basil). *Indian Journal of Experimental Biology* 13 (5) 520-521.

Raschke K. (1976) How stomata resolve the dilema of opposing priorities. *Philosophical Transactions of the Royal Society London* B 273 551-560.

Raymond C.A., Harwood C.E. and Owen J.V. (1986) A conductivity method for screening populations of Eucalypts for frost damage and frost tolerance. *Australian Journal of Botany* 34 377-393.

Retamar J.A. (1986) Essential oils from aromatic species. In: On Essential Oils. pp. 131-135. Ed: Verghese J., R.T.Reddlar & Sons (Ekm.), India.

Richards R.A. and Thurling N. (1978) Variation between and within species of rape-seed (*Brassica campestris* and *B. napus*) in response to drought stress III. Physiological and physiochemical characters. *Australian Journal of Agricultural Research* 29 491-501.

Roderick W.R. (1966) Current ideas on the chemical basis of olfaction. *Journal of Chemical Education* 43 (10) 510-520.

Scheffer J.J.C., Koedam A., Schusler M.I.I.W. and Baerheim Svendsen A. (1977) Improved gas chromatographic analysis of naturally occurring oxygenated monoterpenes following prefractionation by liquid-solid chromatography. *Chromatographia* 10 (11) 669-677.

Scora R.W., Kumamoto J., Horner P.F. and Hollenberg J.L. (1984) Ontogenetic variation and diurnal study in the composition of essential oil in *Artemisia douglasiana*. *Journal of Natural Products* 47 (2) 279-284.

Sharp W.R., Evans D.A. and Ammimato P.V. (1984) Plant genetic engineering: designing crops to meet food industry applications [review]. *Food Technology* 38 (2) 12-119.

Slater C.A. and Watkins W.T. (1964) Citrus essential oils (IV). Transformations of lime oil. *Journal of Science Food and Agriculture* 15 (10) 657-664.

Small B.E.J. (1981) Effects of plant spacing and season on growth of *Melaleuca alternifolia* and yield of tea tree oil. *Australian Journal of Experimental Agriculture and Animal Husbandry* 21 439-191.

Smith S.L. (1987) Possibilities, limitations and future developments in GC-FTIR analysis of essential oils. In: Capillary gas chromatography in essential oil analysis. pp. 367-385. Eds: Sandra P. and Bicchi C., Huethig, Heidelberg.

Snow M.D. and Tingey D.T. (1985) Evaluation of a system for the imposition of water stress. *Plant Physiology* 77 602-607.

Sobrado M.A. and Turner N.C. (1983) A comparison of the water relations characteristics of *Helianthus annuus* and *Helianthus petiolaris* when subjected to water deficits. *Oecologia* 58 309-313.

Southwell I.A. and Armstrong J.A. (1980) Essential oils of the genus *Zieria* (Rutaceae). *VIIIth International Congress Of Essential Oils, Cannes*. 323-337.

Spomer L.A. (1985) Techniques for measuring plant water. *HortScience* 20 (6) 1021-1038.

Squire R.O. and Edgar J.G. (1975) The response of *Eucalyptus obliqua* regrowth to release in irregular stands of mixed eucalypts in west-central Victoria. *Forests Commission Victoria Research Branch Report* 67 pp.22.

Strack D., Proksh P. and Gulz P.-G. (1980) Revesed phase high performance liquid chromatography of essential oils. *Naturforschung* 35 c 675-668.

Sturm W. (1978) Molecular structure and odour. *Household and Personal Products Industry* 15 (10) 78-82.

Suga T., Shishibori T., Kosela S. and Sood V.K. (1975) The neutral volatiles of *Boenninghausenia albiflora*. *Phytochemistry* 14 308-309.

Tamara Y., Nakamura S., Fukui H. and Tabata M. (1984) Comparison of *Stevia* plants from seeds, cuttings and stem cultures for growth and sweet diterpene glucosides. *Plant Cell Reports* 3 (5) 180-182.

Tetenyi P., Kaposi P. and Hethelyi E. (1975) Variations in the essential oils of *Tanacetum vulagare*. *Phytochemisty* 14 (7) 1539-1544.

Tingey D.T. and Stockwell C. (1977) Semipermeable membrane system for subjecting plants to water stress. *Plant Physiology* 60 58-60.

Turner N.C. (1987) The use of the pressure chamber in studies of plant water status. *Proceedings of the International Conference on Measurement of Soil and Plant Water Status. Plants.* 2 13-24.

Vandemeer J. (1984) Plant competition and the yield-density relationship. *Theoretical Biology* 109 393-399.

Vernin G., Petitjean M., Metzger J., Fraisse D., Suon K.N. and Scharff C. (1987) Gas chromatography-mass spectometry of essential oils. Part 1. Computer matching techniques. In: Capillary gas chromatography in essential oil analysis. pp. 287-328. Eds: Sandra P. and Bicchi C. Huethig., Heidelberg.

Viani R., Bricout J., Marion J.P., Muggler-Chavan F., Reymond D. and Egli R.H. (1969) Sur le composition de l'arome de tomate. *Helvetica Chimica Acta* 52 (4) 887-891.

Walker R.R. (1971) Variations in the levels of indole-3-acetic acid and gibberellic acid in the young expanding leaves of toamto plants (*Lycopersicon esculentum* Mill.) subjected to moisture stress. Honours Thesis, University of Tasmania.

Weber J.C., Stettler R.F. and Helman P.E. (1985) Genetic variation and productivity of *Populus trichocarpa* and its hybrids I. Morphology and phenology of 50 native clones. *Canadian Journal of Forest Research* 15 (2) 376-383.

Weiser C.J. (1970) Cold resistance and injury in woody plants. *Science* 169 1269-1278.

Wenninger J.A., Yates R.L. and Dolinsky M. (1967) High resolution infra-red spectra of some naturally occurring sesquiterpene hydrocarbons. *Journal of the A.O.A.C.* 50 (6) 1313-1335.

Werker E. Putievsky E and Ravid U. (1985) The essential oils and glandular hairs in different chemotypes of *Origanum vulgare* L. *Annals of Botany* 55 793-801.

Wood H. and Degabriele R. (1985) Genetic variation and phenotypic plasticity in populations of Paterson's Curse (*Echium plantagineum* L.) in south-eastern Australia. *Australian Journal of Botany* 33 677-685.

Wrigley J.W. (1976) Selection of Australian plant material. *Growing Australian Native Plants Conference Proceedings* Namoi Regional Office, University of New England 42-45.

Yokouchi Y., Hijikata A. and Ambe Y. (1984) Seasonal variation of monoterpene emission rate in a pine forest. *Chemosphere* 13 (2) 255-259.

Yoshihara K., Ohta Y., Sakai T. and Hirose Y. (1969) Germacrene-D. A key intermediate of cadinene group compounds and bourbonenes. *Tetrahedron Letters* 27 2263-2264.

Zalkow L.H., Baxter J.T., McClure R.J. and Gordon M.M. (1980) A phytochemical investigation of *Bothriochloa intermedia*. *Journal of Natural Products* 43 (5) 598-608.

Zdero C., Bohlmann F. and Scott R. (1987) Germacranolides, guaianolides and eudesmanolides from *Greenmanniella resinosa*. *Phytochemistry* 26 (7) 1999-2006.

Zohary D. and Spiegel-Roy P. (1975) Beginnings of fruit growing in the Old World. *Science* 187 319-327.

VII. APPENDICES

APPENDIX A

ANOVA OF GLAND SIZE DATA FOR THREE CLONES

EP

Source	df	SS	MS	F-test	p value
Between subjects	8	1.155e-4	1.444e-5	0.643	0.7351
Within subjects	27	0.001	2.247e-5		
treatments	3	3.366e-4	1.122e-4	9.974	0.0002
residual	24	2.700e-4	1.125e-5		
Total	35	0.001			

GL

Source	df	SS	MS	F-test	p-value
Between subjects	8	3.580e-5	4.475e-6	0.048	0.9999
Within subjects	27	0.003	9.290e-5		
treatments	3	0.002	0.001	30.723	0.0001
residuals	24	0.001	2.159e-5		
Total	35	0.003			

PP

Source	df	SS	MS	F-test	p value
Between subjects	8	9.013e-5	1.127e-5	0.521	0.8302
Within subjects	27	0.001	2.163e-5		
treatments	3	3.522e-4	1.174e-4	12.160	0.0001
residuals	24	2.317e-4	9.655e-6		
Total	35	0.001			

APPENDIX A

COMPARISONS BETWEEN CLONES USING GLAND SIZE DATA

CLONE PAIR	SCHEFFE F-TEST
EP12/EP27	0.359
EP12/EP26	0.043
EP12/EP44	7.834*
EP27/EP26	0.154
EP27/EP44	4.839*
EP26/EP44	6.719*
GL25/GL49	0.361
GL25/GL43	0.034
GL25/GL32	22.653*
GL49/GL42	0.173
GL49/GL32	17.294*
GL42/GL32	20.931*
PP 6/PP22	7.734*
PP 6/PP10	0.200
PP 6/PP34	6.476*
PP22/PP10	5.449*
PP22/PP34	0.0560
PP10/PP34	4.404*

* significant at $p > 0.05$

APPENDIX B

NUMBER OF ESSENTIAL OIL GLANDS PER SQUARE MILLIMETER OF ADAXIAL LEAF SURFACE FOR SELECTED OLEARIA SP. CLONES

CLONE	MEAN NO. GLANDS/mm^2	S.E.
GL 11	20.75	10.53
GL 24	26.50	2.36
GL 25	24.00	6.01
GL 32	56.67	11.67
GL 33	23.50	3.62
GL 42	20.67	8.21
GL 49	68.25	7.28
PP 6	55.75	9.55
PP 7	37.75	4.17
PP 9	20.75	1.55
PP 10	51.50	8.70
PP 12	72.00	6.31
PP 15	53.75	2.39
PP 18	161.00	1.00
PP 22	29.00	1.00
PP 27	90.75	7.69
PP 34	135.75	19.29
PP 39	92.67	5.36
PP 45	47.50	12.06
EP 7	48.28	17.99
EP 12	119.50	32.56
EP 15	75.00	9.37
EP 26	53.75	7.93
EP 27	67.50	17.54
EP 29	99.25	19.93
EP 37	80.25	13.29
EP 41	75.00	5.07
EP 44	71.50	12.18
EP 46	94.00	8.13
EP 49	96.00	6.42
MW	27.00	3.61

APPENDIX C

HPLC REPORT TABLES

Known components are indicated.

a

GREAT LAKE

No.	RT	AREA	CONC	
1	2.335	292777	0.3670	
2	2.605	305667	0.3569	
3	2.741	255256	0.3237	
4	2.958	766777	0.9525	
5	3.678	272576	0.3420	
6	4.705	194210	0.2434	
7	5.365	1866577	2.3402	
8	7.211	255755	0.2434	
9	9.241	230615	0.2590	
10	10.358	241099	0.3022	
11	12.198	197070	0.2470	3
12	12.538	231195	0.2598	4
13	14.515	277253	0.3475	5
14	15.091	221948	0.2782	
15	15.448	1031965	1.2935	11 12
16	16.361	1264395	1.5850	
17	16.979	563405	0.7062	
18	17.531	301511	0.3793	
19	17.748	333348	0.4178	
20	18.318	3646507	4.4812	
21	19.471	2532559	3.1747	
22	20.305	317299	0.3977	
23	20.615	376151	0.4715	
24	21.088	3841162	428152	
25	21.541	320735	0.4020	
26	22.295	312603	0.3918	
27	22.758	2153355	2.6994	
28	23.541	4005956	5.0218	
29	25.035	244178	0.3060	
30	25.878	487052	0.6105	
31	26.238	216511	0.2739	
32	26.598	216121	0.2709	
33	27.238	174212	0.2193	
34	27.761	249945	0.3133	
35	29.048	898119	1.1258	1 2
36	30.395	165427	0.2073	
37	30.975	163812	0.2053	
38	31.575	313290	0.3927	
39	32.508	417078	0.5228	
40	33.521	432227	0.5418	
41	34.568	190769	0.2391	
42	36.128	356451	0.2466	
43	36.128	356451	0.4468	
44	37.529	17893738	22.4313	8
45	38.521	2663379	3.3387	
46	39.995	2336723	2.9292	7
47	40.915	16961542	21.2627	6
48	42.368	509103	0.6382	
49	43.168	470176	0.4640	
50	44.168	1422665	1.7834	
51	44.741	2790109	3.4976	
52	46.215	935463	1.1726	
53	46.988	1189575	1.4912	
54	47.581	215916	0.2706	
55	49.775	477157	0.5981	
56	51.951	486441	0.6097	
57	53.635	158557	0.1987	
TOTAL		79771178	100.0000	

b

MOUNT WELLINGTON

No.	RT	AREA	CONC	
1	2.336	342543	0.3166	
2	2.610	333556	0.3053	
3	2.750	314740	0.2909	
4	2.970	909355	0.8405	
5	5.343	2135125	1.9762	3
6	12.790	180793	0.1671	4
7	14.603	364599	0.3372	5
8	15.023	460375	0.4255	
9	15.403	4739421	4.3805	11 12 1:
10	15.843	425610	0.3961	
11	16.296	685093	0.6332	
12	16.863	296650	0.2742	
13	17.203	323750	0.2992	
14	17.476	309496	0.2860	
15	17.756	317790	0.2937	
16	18.450	2015705	1.8630	
17	19.476	2721555	2.5157	
18	20.990	3700621	3.4204	
19	21.776	484275	0.4476	
20	22.110	346622	0.3203	
21	22.890	5511440	5.0941	
22	24.030	1509903	1.3955	
23	25.630	1394302	1.2857	
24	26.603	655656	0.6057	
25	27.636	171765	0.1557	
26	29.010	515533	0.4795	1 2
27	30.656	200125	0.1749	
28	31.576	220154	0.2035	
29	32.723	259136	0.2395	
30	33.710	1385575	1.2609	
31	34.423	1713373	1.5836	
32	34.976	1251201	1.1564	
33	36.036	1942508	1.7957	
34	37.436	24048374	22.2276	8
35	38.410	3365009	3.1102	
36	39.816	21142754	19.5420	7
37	40.543	9212936	8.5154	6
38	42.463	373217	0.3449	
39	42.963	1000046	0.9243	
40	44.176	4176354	3.8601	
41	44.696	1456435	1.3449	
42	44.923	2346577	2.1710	
43	45.976	938473	0.8674	
44	47.016	1365023	1.2531	
45	47.950	273554	0.2531	
46	52.170	313063	0.2531	
TOTAL		109191407	100.0000	

c

PARADISE PLAINS

No.	RT	AREA	CONC	
1	2.311	250560	0.3493	
2	2.588	254343	0.3546	
3	2.731	214064	0.2984	
4	2.961	765957	1.0680	3 4
5	5.485	1768867	2.4665	5
6	6.398	150782	0.2102	
7	9.061	223508	0.3116	
8	10.211	628224	0.8760	
9	14.441	166984	0.2328	
10	14.998	210605	0.2511	
11	15.365	1283902	1.79027	11 12
12	16.238	658465	0.9151	
13	16.821	543872	0.7571	
14	17.441	284612	0.3963	
15	17.765	191525	0.2674	
16	18.361	5467849	7.6244	
17	19.455	2007462	2.7992	
18	20.248	309811	0.4320	
19	20.521	403346	0.5624	
20	21.011	5398741	7.1097	
21	21.795	271522	0.3786	
22	22.178	273653	0.3815	
23	22.928	1591198	2.2157	
24	23.978	1621407	2.2609	
25	25.821	697521	0.9730	
26	26.629	330787	0.4612	1 2
27	29.081	225500	0.3190	
28	32.808	215016	0.2998	
29	33.841	415108	0.5788	
30	36.261	294045	0.4100	
31	37.528	22399694	31.2343	8
32	38.535	3513753	4.8996	
33	40.048	3686301	5.1402	7
34	41.021	3578915	4.9904	6
35	42.655	272322	0.3797	
36	42.855	355822	0.4961	
37	44.381	2481023	3.4595	
38	44.881	4546919	6.3402	
39	46.368	847856	1.1822	
40	47.221	2161802	3.0144	
41	47.835	471795	0.6579	
42	50.155	585464	0.8163	
TOTAL		71714908	100.0000	

APPENDIX C cont.

d

BUCKLAND

No.	RT	AREA	CONC	
1	2.331	211116	0.1592	
2	2.578	171200	0.1534	
3	2.718	168427	0.1515	
4	2.931	646407	0.5795	
5	5.325	2013211	1.8050	
6	6.815	433707	0.3858	
7	9.135	339135	0.3040	
8	10.258	427923	0.3836	3
9	12.211	262834	0.2356	4
10	12.735	318972	0.2559	5
11	13.348	172357	0.1545	
12	14.018	458538	0.4111	
13	14.408	200368	0.1796	
14	14.585	250000	0.2241	
15	15.018	381399	0.3419	
16	15.415	1192538	1.0692	11 12
17	16.278	1097054	0.9836	
18	16.925	759340	0.6808	
19	17.481	381616	0.3421	
20	17.805	494686	0.4435	
21	18.371	3687141	3.4851	
22	19.471	3166073	2.8386	
23	20.951	1276672	1.1446	
24	21.051	560738	0.5027	
25	21.515	1077520	0.9660	
26	22.138	414912	0.3720	
27	22.938	2302710	2.0645	
28	23.688	621108	0.5568	
29	24.228	361492	0.3241	
30	24.628	375175	0.3363	
31	24.985	184357	0.1652	
32	25.418	473918	0.4249	
33	26.258	263072	0.2358	
34	26.761	318884	0.2859	
35	27.288	368950	0.3307	
36	27.781	565643	0.5071	
37	28.801	504097	0.4519	1
38	29.615	183089	0.1641	
39	30.215	269520	0.2416	2
40	31.175	286345	0.2567	
41	32.028	454435	0.4074	
42	32.921	539688	0.4836	
44	34.595	370475	0.3321	
45	35.228	265519	0.2380	
46	35.888	336595	0.3017	
47	36.595	704874	0.6319	
48	37.635	15584325	13.9728	8
49	37.735	18536828	16.6200	
50	38.588	4060072	3.6402	
51	40.081	14444671	12.9510	7
52	41.095	4443638	3.9541	6
53	42.675	598237	0.5363	
54	43.275	1129166	1.0124	
55	44.435	1760151	1.5781	
56	45.068	9339906	8.3741	
57	46.201	1094546	0.9813	
58	47.355	5923022	5.3105	
59	47.995	1257048	1.1270	
60	50.735	188651	0.1691	
61	52.375	1652433	1.4815	
62	53.428	314711	0.2521	
TOTAL		111533198	100.0000	

e

ELEPHANT PASS

No.	RT	AREA	CONC	
1	2.415	156724	0.2096	
2	2.641	226609	0.3031	
3	2.778	162579	0.2174	
4	2.971	609924	0.8159	
5	4.498	2205072	2.9498	
6	5.038	151835	0.2031	
7	5.318	353609	0.4730	
8	6.031	153072	0.2047	
9	6.781	198366	0.2653	
10	6.945	257122	0.3439	
11	8.911	177995	0.2381	
12	9.278	252344	0.3375	
13	10.391	434745	0.5815	
14	10.978	376097	0.5031	
15	11.718	207009	0.2769	
16	12.411	177586	0.2375	
17	12.878	495597	0.6629	
18	13.345	450268	0.6023	
19	13.781	1819305	2.4324	3
20	14.658	620570	0.8301	4
21	15.565	493051	0.6595	5
22	16.211	223522	0.2990	
23	16.645	752166	1.0062	11 12
24	17.308	1399254	1.8718	
25	18.105	559862	0.7490	
26	18.375	1102320	1.4746	
27	19.085	233503	0.3123	
28	19.445	736648	0.9854	
29	20.128	1379772	1.8444	
30	20.838	177658	0.2376	
31	21.258	507497	0.6789	
32	22.021	2477641	3.3144	
33	23.138	1688847	2.2592	
34	24.298	237056	0.3171	
35	24.868	956782	1.3200	
36	25.271	575517	0.7698	
37	26.238	165630	0.2215	
38	27.988	950929	1.2721	1 2
39	32.488	706071	0.9445	
40	34.681	151904	0.2032	
41	36.148	14192433	18.9859	8
42	37.041	16227479	21.7083	7
43	38.481	5475473	7.3248	
44	39.135	10441649	13.9683	6
45	42.601	1053364	1.4091	
46	43.708	1307743	1.7494	
47	44.575	584410	0.7817	
48	45.755	449661	0.6015	
TOTAL		74752278	100.0000	

f

EAGLEHAWK NECK

No.	RT	AREA	CONC	
1	2.595	176110	0.2846	
2	2.961	430635	0.6959	
3	5.278	1320039	2.1332	
4	9.018	201010	0.3248	3 4 5
5	15.058	460028	0.7434	
6	15.665	290077	0.4687	11 12
7	15.921	255597	0.4130	
8	16.498	315636	0.5100	
9	17.431	310417	0.5016	
10	18.045	1417816	2.2912	
11	19.125	1455917	2.3525	
12	20.311	204677	0.3307	
13	20.705	325094	0.5253	
14	21.131	157844	0.2550	
15	22.501	648392	1.0475	
16	23.665	390269	0.6306	
17	25.355	1401052	2.2642	
18	26.695	172959	0.2795	
19	28.085	165876	0.2650	1 2
20	31.961	379202	0.6125	
21	32.785	392415	0.6341	
22	34.855	156793	0.2533	
23	36.455	24037552	35.9461	8
24	37.341	1817559	2.9672	
25	39.728	14567005	23.5411	7
26	39.615	3980963	6.4334	6
27	41.158	178257	0.2881	
28	41.765	369393	0.5969	
29	42.705	741850	1.1959	
30	43.375	2541263	4.1065	
31	44.621	794751	1.2843	
32	45.501	1556821	2.5159	
33	50.575	265489	0.4290	
TOTAL		61878660	100.0000	

APPENDIX D
MOISTURE STRESS OIL QUALITY
PERCENTAGE COMPOSITION

PEG LEVEL & REP NO.	PEAK RETENTION TIME (min)						
	4.73	5.86	14.97	16.23	16.56	17.15	17.21
4501	0.622	1.288	1.241	4.311	7.499	3.576	2.919
4502	1.540		2.441	9.791	14.042		
4503	0.911	0.505	2.274	2.214	12.616		
4504	1.274	0.647	0.989	5.386	11.911		
3231	0.751		2.588	4.144	8.839	2.855	0.451
3232	0.689	0.332	1.162	7.211	14.391	1.158	0.871
3233	0.819	0.627	1.113	4.625	13.812	1.792	1.112
3234	0.528		1.827	5.067	7.904		
1952	0.613	0.754	2.218	8.534	15.347	2.543	2.258
1953	0.349	0.327	0.718	2.394	7.484	1.077	0.685
1954	1.392		2.851	15.028	16.348		
751	0.469		2.263	4.131	8.057	1.201	3.217
752	0.489	0.574	2.442	5.115	10.617	3.339	2.554
753	0.165	0.241	1.216	1.419	3.521	1.496	1.021
754	0.492	0.922	1.283	3.647	6.075	2.425	2.043
01	0.931		3.617	6.503	12.947	0.245	0.391
02	1.167	1.509	2.098	4.397	11.663	4.575	3.252
03	0.689	0.219	1.828	6.919	8.901	0.407	0.107
04	0.707	0.423	0.641	7.741	12.765	0.244	0.319

APPENDIX E

ANOVA OF PERCENTAGE OIL YIELD FROM OUSE AND BUSHY PARK OLEARIA CLONES; BULK AND SMALL SCALE EXTRACTIONS

Source	d.f.	S.S.	M.S.	F-test	p value
Between	11	8.461	0.769	0.229	0.9938
Within	36	120.698	3.353		
treatments	3	90.859	30.286	33.495	0.0001
residual	33	29.839	0.904		
Total	47	129.159			

Comparisons: R	Scheffe F-test
Site vs. Clone R	8.847 *
Site vs. % Yield Bulk	4.361 *
Site vs. % Yield Lab. Scale	3.825 *
Clone vs. % Yield Bulk	25.632 *
Clone vs. % Yield Lab. Scale	24.307 *
% Yield Bulk vs. % Yield Lab.Scale	0.018

* significant at $p \geq 0.05$

APPENDIX F

ANOVA OF FRESH WEIGHT OF HARVESTED MATERIAL FOR DISTILLATION FROM BUSHY PARK AND OUSE

Source	d.f.	S.S.	M.S.	F-test	p value
Between	11	534.457	48.587	0.325	0.972
Within	24	3588.813	149.534		
treatments	2	2688.500	1344.250	32.848	0.0001
residual	22	900.313	40.923		
Total	35	4123.270			

Comparisons:	Scheffe F-test
Clone vs. Site	0.293
Clone vs. Weight	21.814 *
Site vs. Weight	27.165 *

* significant at $p \geq 0.05$

APPENDIX G

COMPARATIVE AREA PERCENTAGE CHEMICAL COMPOSITION OF STEAM DISTILLED OILS FROM SIX OLEARIA CLONES

(Samples collected August 1988, Ouse)

Component	Area Percentage					
	MW	PP	EP	EN	BU	GL
α-pinene	0.29	1.49	6.91	1.02	0.28	1.88
β-pinene	1.44	2.55	2.18	2.05	3.84	0.24
1,8-cineole	0.65	1.69	1.31	ns	ns	1.19
linalool	0.49	26.80	4.92	7.72	ns	0.38
α-terpineol	ns	2.79	0.87	0.68	ns	ns
caryophyllene	0.17	2.55	2.41	2.09	5.94	40.10
germacrene-D	13.45	ns	8.34	9.04	4.77	ns
bicyclogermacrene	22.52	7.80	4.37	26.02	35.60	15.61
elemol	ns	4.40	ns	ns	ns	ns
spathulenol	5.93	ns	0.91	8.06	12.86	6.81
γ-eudesmol	3.02	7.33	ns	ns	ns	ns
β-eudesmol	6.34	11.01	36.55	ns	ns	1.97
α-eudesmol	3.59	7.26	ns	ns	ns	ns